Genomic analysis of hybridization between the spruce budworm species *Choristoneura fumiferana, C. occidentalis*, and *C. biennis* (Lepidoptera: Tortricidae)

by

Bryan Thomas Michaelo Brunet

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Systematics and Evolution

Department of Biological Sciences
University of Alberta

© Bryan Thomas Michaelo Brunet, 2014

Abstract

The spruce budworm (*Choristoneura fumiferana*) species complex is a destructive group of insect defoliators of North American conifers that have a remarkable propensity to hybridize *ex situ* despite little evidence for hybridization in nature. Introgression between species is increasingly identified as a source for adaptive genetic variation. Hybridization between these species could have important implications in the management of forest resources. In addition, hybrid zones can reveal important insights about the evolutionary processes that underlie species divergence. In this thesis, I use a combination of traditional and more recent molecular approaches to investigate a putative hybrid zone between three spruce budworm species, *C. fumiferana*, *C. biennis*, and *C. occidentalis,* that straddles the Rocky Mountains of Alberta and British Columbia. Using mitochondrial COI DNA and nuclear microsatellite gene markers, I identify an outbreaking population of *C. occidentalis* in southwestern Alberta occurring on alternative host trees in the area (Chapter 2). This work confirmed the presence of hybrid individuals with *C. fumiferana* in the area and noted similarities in the phenology of budworms on Douglas-fir and a hybrid spruce species. I then turned to genomic methods and exploited a large EST sequence resource to develop a suite of EST-based microsatellite loci suitable for genetic analysis of *C. fumiferana* (Chapter 3). After that, by using an even newer genome-wide marker discovery approach called 'Genotyping-By-Sequencing (GBS)', I investigated the population structure and gene flow between the three spruce budworm species that interact along the northern Rocky Mountains (Chapter 4). I concluded that recent hybridization between eastern (*C. fumiferana*) and western *(C. biennis/C. occidentalis*) populations is not extensive, although there is evidence for historical gene flow. However, whereas three species were expected, we find overwhelming support for a two-species (eastern and western) dynamic in this region.

Populations of *C. biennis* and *C. occidentalis* identified on the basis of life history traits were only weakly supported as genetically identifiable subpopulations within a western lineage. I compare the patterns of differential introgression between *C. biennis* and *C. occidentalis* (recent divergence) with those between *C. fumiferana* and *C. occidentalis* (deep divergence) to determine if there was correspondence among the loci subject to selection, as well as the modes of selection in each comparison (Chapter 5). Clines between the deeply diverged species were steep and highly concordant across loci, suggesting strong selection against hybrids, whereas those between the recently diverged species were linear and gradual. However, directional selection was found to be the predominant mode of selection in each comparison. Finally, I developed a preliminary linkage map for an outbred hybrid backcross between *C. biennis* and *C. occidentalis* to assist in establishing linkage relationships between unassembled scaffolds in a spruce budworm genome assembly that is currently in development (Chapter 6).

Ultimately, this work highlights the usefulness of genome-wide approaches for marker discovery and development. Where hybridization between *C. fumiferana* and the western species was presumed to be rampant, these results reveal that it is not. On the other hand, these results also call into question the validity of *C. biennis* as a species distinct from *C. occidentalis*. Overall, this work provides a foundation for future work to elucidate the mechanisms responsible for maintaining reproductive isolation between these species, as well as the genomic architecture of species differences.

Preface

A version of Chapter 2 of this thesis has been published as: Brunet, B.T.M., Hundsdoerfer, A.E., and F.A.H. Sperling (2013) Identification and ecological characterisation of Choristoneura occidentalis (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada. The Canadian Entomologist 145: 521-528. I contributed the experimental design and was responsible for data collection, analysis, and manuscript preparation. The experimental design was largely crafted by Anina Hundsoerfer, and she also contributed to data collection and manuscript editing. Felix Sperling contributed to organizing logistics for data collection and to manuscript revisions.

A version of Chapter 3 of this thesis has been published as: Brunet, B.T.M., Doucet, D., Sturtevant, B.R., and F.A.H. Sperling (2013) Characterization of EST-based SSR loci in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Conservation Genetics Resources, 5: 541-544. I contributed the design of the study, microsatellite development and data analysis, and manuscript preparation as well as to some sample collection. Daniel Doucet provided the expressed sequence tag libraries used in microsatellite discovery and also contributed to manuscript editing. Brian Sturtevant provided spruce budworm samples and contributed to manuscript editing. Felix Sperling helped in the design of the study and manuscript revisions. An additional manuscript using these microsatellite markers to survey population structure within *C. fumiferana* in the Border Lakes Landscape area of northern Minnesota and Ontario is under peer-review for publication (James *et al*. in review). For this work, I contributed to microsatellite data collection, analysis, and writing and editing of the manuscript.

This thesis is dedicated to my

wife, Staci, and our son, Luc.

Acknowledgements

I have many people to thank for encouragement and support throughout my graduate studies. First and foremost, I would like to thank my supervisor Felix Sperling for his support and patience over the years, and for introducing me to such a wonderful environment to work in. I have truly enjoyed my time in his lab, and will always hold this time in my life very dearly.

To my fellow lab members, past and present, Julian Dupuis, Jasmine Janes, Jason Dombroskie, Ben Proshek, Christianne McDonald, Christi Jaeger, Sarah Leo, Sarah McPike, Winnie Lam, Wes Hunting, Lisa Lumley, Thomas Simonsen, Marie Djernas, Patrick James, Yisu Li, Giovanny Fagua, Heather Bird, and Marla Schwarzfeld, thank you for making this experience so enjoyable. I'm also grateful for friendships with Boyd Mori, Adam Blake, and Alex Smith. I am grateful to have met such a fantastic group of people while at the University of Alberta. I would also like to mention Gary Anweiler for his assistance in collection, as well as his company during Christmas bird counts.

I would like to thank family located here in Alberta, home in Ontario, and elsewhere. My mother, Debbie, and father, Tom, were supportive of this adventure from the start. Without either of them, the trip to Edmonton would not have been possible. I can honestly say that if my dad and brother, Travis, had not accompanied me on the drive across the country, I'd probably be stuck in Russel, Manitoba or North Battleford, Saskatchewan. I'd like to thank my wife, Staci, who has made many sacrifices, including surrendering her home to six-legged critters first and now more recently, a four-legged one, and yet continues to be a constant source of enthusiasm, encouragement, and love. She also allowed me to go bug-collecting on our honeymoon!

Thanks also to Anina Hundsdoerfer who played a large role in directing my research toward spruce budworms. She also played an integral role in collecting samples and securing funds for an Alberta Innovates project that I am currently involved in.

I would like to thank the members of my supervisory committee, Paul Stothard and David Coltman, for their guidance and ideas. Jan Volney, and Francis Yeh also participated in my candidacy examination committee. Thanks also to Sean Rogers and Andreas Hamann for agreeing to be on my defense committee. Not only are you all inspirational scientists, but also great teachers and leaders.

Many of the resources/tools I used were generously provided by others: Patricia Beldade and Anthony Long provided scripts for microsatellite development; Daniel Doucet made available the expressed sequence tag libarires; Paul Stothard provided assistance in the post-processing and analysis of ESTs, and offered the use of his personal computing cluster; Roger Levesque and Michel Cusson provided access to the spruce budworm reference genome; Brian Boyle and Jerome Laroche provided support with GBS analysis; Jerome and Halim Maaroufi provided synteny data; Vince Nealis provided the hybrid backcrossed mapping family for Chapter 6; and Pasi Rastas provided support and scripts for the use of Lep-Map.

Funding for this research was provided by a Government of Alberta Queen Elizabeth II Graduate Scholarship to myself, a Natural Sciences and Engineering Research Council of Canada Discovery Grant to Felix Sperling, an Alberta Innovates BioSolutions grant to Felix Sperling, and grants from the NRCAN Genomics Research and Development Initiative and the USDA National Research Initiative to coauthors on Chapter 3.

Table of Contents

Abstract ... ii

Preface ... iv

Dedication ... v

Acknowledgements ... vi

List of Tables ... xv

List of Figures ... xvi

List of Abbreviations ... xx

Chapter 1: General Introduction ... 1

Life History and Reproductive Barriers ... 3

Premating Barriers to Gene Flow ... 4

Postmating Barriers to Gene Flow ... 7

Thesis Objectives ... 8

Literature Cited ... 14

Chapter 2: Identification and ecological characterization of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada ... 27

Summary 27

Introduction 27

Methods 30

Study sites and sampling design 30

Budworm species identity 30

Results 31

Species identity 32

Discussion 33

Literature Cited 41

Chapter 3: Characterization of EST-based SSR loci in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) 47

Summary 47

Main Text 47

Literature Cited 53

Appendix 3-1 56

Chapter 4: Two's company, three's a crowd: population structure and asymmetry in a three-way hybrid zone between spruce budworm species in the Canadian Rocky Mountains 58

Introduction 58

Methods 62

Sample collection 62

DNA extraction and 'Genotyping-By-Sequencing' 63

SNP discovery and genotyping 64

Genetic diversity and differentiation 66

Population structure 66

Hybrid identification 67

Patterns of gene flow 68

Results 69

Sample collection and Genotyping-By-Sequencing 69

Genetic diversity and genetic differentiation 70

Population structure 71

Hybrid identification 72

Patterns of gene flow 73

Discussion 74

Species status of C. fumiferana, C. biennis and C. occidentalis 74

Patterns of hybridization 75

Processes contributing to divergence and speciation 76

Limitations 79

Conclusions 83

Literature Cited 96

Appendix 4-1 109

Appendix 4-2 120

Appendix 4-3 122

Chapter 5: Differential introgression identifies speciation genes at varying levels of phylogenetic divergence within the spruce budworm, *Choristoneura fumiferana*, species complex 139

Introduction 139

Methods 143

Sampling 143

Markers 143

Candidate loci under natural selection 144

Admixture analysis and genomic clines 145

Geographic clines 147

Results 148

Distribution of ancestry coefficients reveals contrasting patterns of admixture 148

Outlier analysis 148

Genomic clines 149

Geographic clines 150

Discussion 151

Patterns of introgression within clines 152

Contrasting patterns of differential introgression at different levels of phylogenetic divergence 156

Conclusions 158

Literature Cited 167

Appendix 5-1 178

Appendix 5-2 192

Appendix 5-3 205

Appendix 5-4 208

Chapter 6: Linkage mapping 210

Introduction 210

Methods 212

Crosses 212

GBS library preparation and sequencing 213

Data processing 214

Identifying chromosomes 215

Chromosomal maps 216

Chromosome homology and synteny with Bombyx mori 217

Results 218

GBS data filtering 218

Linkage mapping 218

Chromosome homology 220

Discussion 221

Literature Cited 232

Appendix 6-1 239

Appendix 6-2 269

Chapter 7: General Conclusions 272

Thesis Summary and Future Directions 272

Development of tools for molecular ecology 272

Spruce budworm hybridization 274

Systematic and taxonomic considerations 277

Additional Future Directions 278

Literature Cited 281

Biography 286

Bibliography 288

List of Tables

Table 2-1: Sample size of larval/pupal and adult (male) *Choristoneura* collections by ordinal date. Parentheses denote adult collections that occurred concurrently with larval collections. ... 37

Table 2-2: *Choristoneura* collection site, method of collection (adult reared from larva or pheromone trapped), and combined genetic profile (microsatellite population /mtDNA *co1* haplotype (sample size)). Previously named haplotypes are as in Sperling and Hickey (1994) and Lumley and Sperling (2010, 2011a, 2011b). 38

Table 3-1: Summary data for 20 EST-SSRs tested on 31 individuals of *Choristoneura fumiferana* from the Border Lakes landscape of northern Minnesota. 52

Table 4-1: Population sample sizes and geographic coordinates. 85

Table 4-2: Mean samples size (N), observed (H_o) and unbiased expected heterozygosity (uH_e), and fixation index (F) across 2277 loci for each population. 87

Table 5-1: Collection locations and sample size. 160

Table 5-2: Summary of cline analyses (genomic and geographic), and genetic differentiation across all loci showing significant non-neutral patterns of introgression. 162

Table 6-1: Mapping family and sequencing information. 225

Table 6-2: Maternal linkage groups with locus density and map lengths. 226

Table 6-3: Chromosome homology between spruce budworm linkage groups and the *Bombyx mori* genome. 228

List of Figures

Figure 1-1. Species distributions of the spruce budworm, *Choristoneura fumiferana*, the western spruce budworm, *C. occidentalis*, and the two-year-cycle spruce budworm, *C. biennis*. Modified from Figure 4-3 in Lumley (2010). 13

Figure 2-1. Southwestern Alberta, Canada sample sites showing larval hosts (hybrid spruce, Douglas-fir), and elevation. Photograph is from Google Maps (https://maps.google.com), with the addition of major highways and location of the study areas within Alberta. 39

Figure 2-2. Developmental progression of *Choristoneura* populations at five sites in southwestern Alberta, Canada. Left side shows observed average instar for each site and the regression line for all sites combined, with two to six for larval instars and seven for pupae. Right side show arcsine square-root transformed cumulative proportion of male moth abundance per collection date at each set with the regression line for all sites combined. The right axis is presented back-transformed. Horizontal axis shows ordinal date. 40

Figure 4-1. Structure results showing A) barplot for individuals assigned to K=2 genetic clusters; B) distribution of mean estimates of the log probability of the data at each value of K; and C) delta K plot resulting from the Evanno *et al*. (2005) method. Individuals in A) are sorted by their ancestry assignment to each cluster at K=2. Results present in B and C show significant support for $K=2$ over larger values of K. 89

Figure 4-2. Geographic distribution of sampled populations showing the populations with admixed individuals between eastern and western spruce budworm genetic clusters. Populations are represented as pie charts indicating the proportion of each population with ancestry assignment (q >= 0.9) to each genetic cluster identified by Structure at K=2. Clusters correspond

with *C. fumiferana,* and a western cluster consisting of *C. biennis* and *C. occidentalis*. Pie chart size corresponds with sample size (*N*) at each location. Reference populations for *C. fumiferana*, *C. occidentalis* and *C. biennis* are labeled next to their corresponding pie charts. Inset shows the location of the sampled area relative to western Canada.. 90

Figure 4-3. Structure results for the hierarchical analysis of substructure within the *K*=2 western genetic cluster consisting of 365 individuals with >95% assignment probability. A) shows the barplot for western individuals assigned to *K*=2 subpopulations; B) the distribution of mean estimates of the log probability of the data at each value of *K*; and C) the delta *K* plot resulting from the Evanno *et al*. (2005) method. Individuals in A) are sorted by their ancestry assignment to each western subpopulation at *K*=2. Results present in B and C show weak support for *K*=2.91

Figure 4-4. Geographic distribution of population substructure within the western genetic cluster showing the populations with admixed individuals between the western subpopulations. Populations are represented as pie charts indicating the proportion of each population with ancestry assignment (*q* >= 0.9) to each of the western subpopulations identified by Structure at *K*=2 when only western individuals are considered (*N*=365). The subpopulations correspond with *C. occidentalis* and *C. biennis* reference populations, respectively. Pie chart size corresponds with sample size (*N*) at each location. Reference populations for *C. occidentalis* and *C. biennis* are labeled next to their corresponding pie charts. The inset shows the location of the sampled area relative to western Canada. 92

Figure 4-5. Discriminant analysis of principal components (DAPC) scatterplot showing the clustering of individuals along the first two principal components. Individuals cluster into three groups that correspond with *Choristoneura fumiferana*, *C. occidentalis*, and *C. biennis* as determined based on clustering with reference populations of each species. 93

Figure 4-6. Triangle plots resulting from two-way hybrid analysis in HIest. Plots show the proportion of markers heterozygous (H_I) for *Choristoneura fumiferana* and *C. occidentalis* alleles (A), *C. fumiferana* and *C. biennis* alleles (B), and *C. occidentalis* and *C. biennis* alleles (C) in each individual plotted against hybrid index (S). 94

Figure 4-7. Asymmetric gene flow model resulting from Migrate-n analyses showing modal estimates (with 95% support limits) of mutation-scaled effective population size (Θ) for *C. fumiferana, C. biennis*, and *C. occidentalis*, as well as the effective number of migrants per generation (N_em, above and below arrows) in each direction between the three species. 95

Figure 5-1: Map of the study area showing collection locations. Reference populations are identified as Edmonton (Ed) and Brightsands Lake (BL) for *C. fumiferana*, Bull River (BR) and Porcupine Hills – South (P2) for *C. occidentalis*, and Morrisey Creek (Mr) and McBride (Mb) for *C. biennis.* The geographic cline between *C. fumiferana* and *C. occidentalis* runs from Mt to BL. Boundaries of the area of hybridization between *C. fumiferana* and *C. occidentalis*, as deduced by geographic clines, are indicated by perforated lines, and the geographic cline centre by a dashed line. 163

Figure 5-2: Distribution of hybrid indices or ancestry coefficients across genomic clines between *C. fumiferana* and *C. occidentalis* (n=461, top), and *C. biennis* and *C. occidentalis* (n=365, bottom). 164

Figure 5-3: Locus-specific genomic and geographic clines. Genomic clines are plotted as the frequency of the *Choristoneura fumiferana* (left) and *C. biennis* (middle) homozygote genotypes against hybrid index (1 = *C. occidentalis*). Geographic clines are plotted as the frequency of *C. fumiferana* alleles against distance from Merritt (BC). 165

Figure 5-4: Correlation of locus-specific geographic cline width (*w*) and centre (*c*) estimates for 114 loci with significant allele frequency differences between *Choristoneura fumiferana* and *C. occidentalis*. 166

Figure 6-1: Spruce budworm linkage map indicating marker positions (cM) along each putative chromosome (left) and homologous regions within the *Bombyx mori* linkage map (right). Locus names at each position are listed in Appendix 6-1. 230

List of Abbreviations

COI – cytochrome oxidase 1 gene

DNA – deoxyribonucleic acid

mtDNA – mitochondrial DNA

EST – expressed sequence tag

GBS – Genotyping-By-Sequencing

SSR – Simple sequence tag

STR – Simple tandem repeat

F1 – Filial 1st generation

F2 – Filial 2nd generation

AESRD – Alberta Environmental and Sustainable Resource Development

WSBW – Western spruce budworm

SBW – spruce budworm

PCR – polymerase chain reaction

SNP – single nucleotide polymorphism

RAD – restriction enzyme associated DNA

AMOVA – analysis of molecular variance

DAPC – discriminant analysis of principal components

LOD – logarithim of odds

HWE – Hardy-Weinberg equilibrium

LD – linkage equilibrium

Chapter 1

General Introduction

Hybridization is increasingly recognized as an important source of adaptive genetic variation in nature (Mallet 2005; Abbott *et al*. 2013). Reproduction between disparate taxa typically results in inviability or infertility of the resulting hybrid progeny due to accumulation of incompatible alleles derived from independent genomic backgrounds (Orr and Turelli 2001; Matute *et al*. 2010; Wang *et al*. 2013). However, hybridization can also generate novel and advantageous genetic combinations. For instance, recombination leading to the alignment of complementary sets of alleles that have been isolated in the pure parental lineages can result in transgressive hybrid phenotypes due to the additive action of these genes (Rieseberg *et al*. 1999). This can result in hybrid progeny being more fit even in parental habitats (Rieseberg *et al*. 1999). In addition, hybridization can result in introgression of beneficial traits or 'adaptive introgression' between species (Arnold and Martin 2009; Dasmahapatra *et al.* 2013; Hedrick 2013). Thus, the study of hybrid zones can reveal important insights into the process of adaptation and speciation.

Hybridization among the conifer-feeding species of *Choristoneura* Lederer, which comprise the spruce budworm, *Choristoneura fumiferana* (Clemens), species complex, has been widely documented (Smith 1953, 1954; Campbell 1958, 1967, Sanders 1977; Liebhold *et al*. 1984; Volney *et al*. 1984; Volney and Liebhold 1985; Sperling and Hickey 1994; Gray *et al*. 1995; Powell and De Benedictis 1995; Shepherd *et al*. 1995; Harvey 1997; Deverno *et al*. 1998; Lumley and Sperling 2011a; Lumley and Sperling 2011b; Brunet *et al*. 2013). Between 8 and 15 species and/or biotypes make up this complex of highly destructive forest pests (Volney and

Fleming 2007; Lumley and Sperling 2011a). In Canada, three spruce-feeding budworn species cause significant economic damage to the forestry industry: the spruce budworm (*C. fumiferana*), the western spruce budworm (*Choristoneura occidentalis* Freeman), and the two-year cycle spruce budworm (*Choristoneura biennis* Freeman) (Nealis 2008).

Historically these species have been treated as part of allopatric eastern (*C. fumiferana*) and western (*C. biennis* and *C. occidentalis*) cohorts (Stehr 1967; Harvey 1985a). The distribution of *C. fumiferana* extends from the maritime provinces of Canada to Alberta, northern British Columbia (BC), Yukon, Northwest Territories, and Alaska. Its southern extent is limited to the United States neighboring the Great Lakes and St. Lawrence Seaway (Lumley and Sperling 2011) (Figure 1-1). In the western cohort, *C. occidentalis* occurs from the Coastal to Rocky Mountains of southern BC, with southern extensions along the Pacific coast to northern California, and along the Rocky Mountain states to Arizona and New Mexico (Lumley and Sperling 2011). Alternatively, the distribution of *C. biennis* is mainly limited to northern BC and the Rocky Mountains of Alberta and BC (Lumley and Sperling 2011). The boundaries between these three species, however, are becoming increasingly blurred as hybrids continue to be identified where their ranges meet (Sperling and Hickey 1994; Shepherd *et al*. 1995; Lumley and Sperling 2011a; Brunet *et al*. 2013). Most evidence for hybridization has come to light using modern deoxyribonucleic acid (DNA) sequencing and genotyping techniques that have allowed researchers to circumvent the cryptic biology of these species (Sperling and Hickey 1994, Shepherd *et al*. 1995, Gray *et al*. 1995; Lumley and Sperling 2011a, 2011b; Brunet *et al*. 2013). Although this is particularly true for budworm species that use similar hosts and share similar sex pheromone chemistry, which may increase the frequency of interspecific interaction in nature, there is a wealth of literature citing weak and incomplete barriers to gene flow between

all species within this species complex (Campbell 1958, 1967; Sanders *et al*. 1977; Harvey 1997).

Life history and Reproductive Barriers

All coniferophagous *Choristoneura* species share a common life history strategy (Nealis 2008). Gravid females oviposit large clusters of eggs on the needles of their hosts (Campbell 1958; Harvey 1977). Upon eclosion, first instar larvae disperse to overwintering locations without feeding, spin hibernacula in which they molt into the second instar, and then enter diapause (Regniere and Fletcher 1983). Within controlled environments it is possible to observe a small fraction of second instar larvae that do not enter this obligate diapause stage, but instead commence feeding and resume larval development (Harvey 1957; Lyon *et al*. 1972). Survival of these larvae in nature, however, is futile as the nutritional content of host trees and climate is unsuitable for larval development (Harvey 1957; Miller 1958). Post-diapause emergence is variable but within a single generation the emergence of a substantial fraction of individuals is synchronized with the flushing of buds on host trees (Volney *et al.* 1983; Lysyk 1989; Volney and Cerezke 1992). Larvae that emerge earlier than bud flush will disperse to alternative food sources, such as staminate cones and the previous year's needles, until the onset of bud flush of the current year's growth (McGugan 1954; Nealis and Regniere 2009), at which point they will transfer to this new growth. Typically, four post-diapause instars will follow in succession; however, developmental polymorphism has been observed (Schmidt and Lauer 1977; Schmidt *et al*. 1977). In addition, some species (*e.g. C. biennis*) enter a second diapause stage during the third larval instar (Harvey 1967; Nealis 2005). Sexual dimorphism in larval development is also

apparent with males typically completing development earlier than females (Smith 1953; McGugan 1954). Following pupation, adults emerge and are capable of dispersing hundreds of kilometres in the search for suitable mates (Greenbank *et al*. 1980; Dobesberger *et al*. 1983). Females mate and oviposit nearly half of their total egg complement prior to dispersal, and predominate in dispersing populations, particularly late in the season (Greenbank *et al*. 1980). However, females will mate multiple times throughout their lifetimes (Greenbank *et al*. 1980).

Premating Barriers to Gene Flow

Reproductive barriers to gene flow between species can be categorized according to the point at which they act between species (Coyne and Orr 2004; Nosil *et al.* 2005): 1) barriers that act prior to mating (premating), 2) those that act after mating but before fertilization (postmating), and 3) those that act after fertilization (postzygotic). Within the spruce budworm species complex each of these barrier groups can take on multiple forms. Premating barriers are perhaps the most effective barriers to reproductive isolation between spruce budworms, but provide only partial resistance to gene flow between species (Sanders 1991), and are primarily a result of ecological and behavioural isolation. As noted earlier, the complex is divided into two largely allopatric cohorts with strong correspondence to distinct (boreal or montane) forest regions (Stehr 1967). Where species are sympatric within each cohort strong affinities for alternative primary host species serves to further isolate species (Smith 1953; Stehr 1967). For instance, within the eastern cohort, sympatric *C. fumiferana* and *C. pinus* primarily use spruce/fir and pine species, respectively (Freeman 1947; Smith1953; Freeman 1967). However, where host species are spatially intermixed, isolation of habitat does not appear to be a significant barrier to

gene flow and hybrid populations of sympatric *Choristoneura* have been identified in both western and eastern cohorts (Volney *et al*. 1983, 1984; Liebhold *et al*. 1984; Liebhold and Volney 1984; Volney and Liebhold 1985; Liebhold 1986; Smith 1954; Deverno *et al*. 1998).

Allochronic isolating barriers, or barriers that minimize interspecifc interaction over temporal rather than spatial scales, may offer some resistance to gene flow. Post-diapause emergence times differ between sympatric species occurring on alternative hosts (Smith 1953), resulting in disparate peak moth flight periods. Temporal isolation of sympatric populations of multiple species of spruce budworms has recently been demonstrated in an isolated forest island habitat (Lumley and Sperling 2011b). Smith (1953) also showed that development of female spruce budworms is also delayed relative to males and suggested that hybridization between sympatric *C. fumiferana* and *C. pinus* would be more likely to occur between female *C. fumiferana* and male *C. pinus*, as the peak flight of the latter species occurs nearly two weeks after that of *C. fumiferana.* However, similar developmental differences are not apparent between two sympatric western species, *C. occidentalis* and *C. retiniana* (Walsingham). In addition, although subtle differences exist between species in the timing of female calling and mating (Sanders 1971a), such differences are unlikely to prevent hybridization (Smith 1954; Sanders 1971a; Harvey 1985a).

Mating behaviour may provide the most effective premating barriers to gene flow. Differences in the primary sex pheromone constituents exist between species and western and eastern geographical cohorts can be divided into species responding to either an aldehyde or acetate/alcohol pheromone blend (Sanders 1971b; Sanders 1977; Harvey 1985a; Powell and De Benedictis 1995). However, sex pheromone chemistry has been shown to be insufficient in maintaining isolation of species with similar host preferences (Liebhold *et al*. 1984; Daterman *et*

al. 1995; De Benedictis *et al*. 1995), and mate-choice experiments have consistently demonstrated free interbreeding among species within the complex (Smith 1954; Campbell 1967; Sanders 1971a; Sanders *et al.* 1977; Harvey 1997). Despite this, conflicting evidence exists for alternate roles of differences in cross-attraction between these species. For instance, Campbell (1967) noted a high degree of sexual aggressiveness in males of *C. fumiferana* compared to those of western spruce-fir feeding *Choristoneura* and choosy females of the same species, and postulated that such asymmetry could lead to introgression from *C. fumiferana* into western genomes. Conversely, Sanders *et al*. (1977) reported that male *C. fumiferana* attraction to conspecific females was decreased in the presence of congeneric females, whereas the same effect was not observed in other species. However, such differences were not observed in a more recent comprehensive analysis of inter-fertile hybrids between all spruce budworm species (Harvey 1997).

It remains to be determined whether interspecific differences in the male reproductive organs (*i.e.* the incidence of spicules on the aedeagus, Dang 1985) result in mechanical isolation, another form of premating barrier. Gray *et al*. (1995) and Shepherd *et al.* (1995) demonstrated a cline in the frequency of spicules in spruce budworm populations from eastern to western Canada, with high frequencies in *C. fumiferana* populations, low frequencies in *C. biennis* populations, and an absence of spicules in *C. occidentalis*. The strong discordance in spicule frequency between populations of eastern and western species suggests that differences in genitalic morphology could limit gene flow between species.

Postmating Barriers to Gene Flow

There are no known postmating barriers, whether pre- or post-zygotic, leading to reproductive isolation among spruce budworms (Smith 1953). Hybrid progeny are readily attainable under controlled environments and are generally fertile among themselves and in successive backcrosses (Sanders *et al.* 1977; Harvey 1997). Although some differences in chromosome structure have been documented in *C. fumiferana* and *C. biennis* (Ennis 1976), it appears that intrinsic isolating barriers resulting from genic (*e.g.* alleles of one species are unsuited to genomic backgrounds of a second species) or chromosomal incompatibilities (*eg.* rearrangements) are unlikely (Shepherd *et al.* 1995; Harvey 1997). This lack in intrinsic post-zygotic barriers probably relates to the close phylogenetic relationships in the complex (Sperling and Hickey 1994, 1995; Bird 2013; Wang *et al*. 2013). The effects of extrinsic postzygotic factors, however, have not been sufficiently explored. The strong concordance between forest regions and isolation observed within the complex might elicit ecological inviability of hybrids in nature (Rundle and Nosil 2005; Nosil 2012). Additionally, the extent of selection against immigrants between species with parapatric distributions is unknown (Nosil *et al.* 2005). However, some evidence suggests that where hybrids do occur in nature, they may experience increased fitness relative to parental populations. Egg weights, for example, are correlated with the survival of larvae (Harvey 1983a, 1983b, 1985b), and differ in F1 females from reciprocal crosses between species (Campbell 1958; Harvey 1997). Female progeny from crosses between male *C. fumiferana* and females of other species result in greater egg weights than females of the opposite configuration (Harvey 1997). Therefore, it seems possible that at least some hybrid combinations could be selectively advantageous under natural circumstances.

Thus, although reproductive barriers to gene flow between coniferophagous species of spruce budworms are known, none is sufficient to completely establish reproduction isolation between these species. As such, hybridization is likely to ensue in areas where these species are sympatric in nature.

Thesis objectives

In this thesis, I examine populations of the spruce budworm species *C. fumiferana, C. biennis,* and *C. occidentalis* in the Rocky Mountains and surrounding areas using a combination of traditional and modern molecular methods. The need to determine the identity and origin of an outbreak population of *Choristoneura* in southwestern Alberta formed the basis of Chapter 2, as the area contains a mix of both boreal and montane conifer species, as well as their hybrids. The area is also the junction between budworms of eastern and western distribution. Therefore, clearcut identification of the populations in this area based upon geography and host use, as is typically done within the spruce budworm species complex (Harvey 1985, Lumley and Sperling 2010) was not possible. Likewise, the development of spruce budworm populations is known to show significant synchronicity with the new growth of foliage on their host trees when the physiological attributes of the host are favorable (Lawrence *et al*. 1997). Consequently, budworm phenology may be affected by development on alternative hosts (Lysyk 1989) and in different locations (Lysyk 1989; Volney and Cerezke 1992). Since the management of budworm populations is primarily through the application of pesticides, such as *Bacillus thuringiensis* var. *kurtaki*, which are most effective against particular stages in budworm development (Cadogan *et al*. 1995), knowledge of phenological differences can help inform

management decisions. Accordingly, in collaboration with Alberta Environment and Sustainable Resource Development (AESRD), I used mitochondrial cytochrome oxidase I (CO1) DNA (mtDNA) and an existing resource of microsatellite genetic markers (Lumley *et al.* 2009) to identify spruce budworm populations from the outbreak area in southwestern Alberta. I then characterized the larval and adult phenology on alternative hosts over the course of an entire active budworm season. This study is now published as Brunet, Hundsdoerfer and Sperling (2013a).

A paucity of genetic markers for the investigation of spruce budworm population structure and hybridization, in combination with a desire to move beyond inefficient traditional molecular techniques for marker development (Zane *et al.* 2002), led me to turn my attention to the exploration and development of microsatellites from existing genomic resources. Through collaboration with a spruce budworm researcher located at the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario, I acquired access to a collection of expressed sequence tag libraries (ESTs). In Chapter 3, I used *in silico* computational methods to discover microsatellite or simple sequence repeat (SSR) markers embedded in these ESTs, and then developed laboratory methods for the practical application of these markers in genetic surveys of spruce budworm populations (primer note now published as Brunet *et al.* 2013b).

In parallel with my development of EST-SSR loci, however, a significant revolution in DNA sequencing technologies was quickly taking hold in the field of population genetics (Ekblom and Galindo 2010; Gardner *et al*. 2011). Novel approaches for genetic marker discovery and genotyping were now able to harness the high-throughput capabilities of next-generation sequencing platforms and bring genome-wide studies of non-model organisms to the forefront of molecular ecology (Davey *et al*. 2011; Davey and Blaxter 2011; Rowe *et al*. 2011).

Consequently, I once again turned my attention to new molecular approaches and adopted a Genotyping-By-Sequencing (GBS) approach (Elshire *et al*. 2011; Poland *et al*. 2012) for Chapters 4-6.

Since hybridization can lead to the spread of advantageous traits from one species into another (Morjan and Rieseberg 2004; Parsons *et al.* 2006; Martin *et al*. 2006), such circumstances could have dire consequences in the management of important forest pests, like spruce budworms. In addition, hybridization has been known to facilitate the range expansion of invasive species (Nolte *et al*. 2009; Scascitelli *et al*. 2010). Prior hypotheses have suggested that the direction of gene flow between eastern and western spruce budworms would be more likely to follow a west-to-east direction on the basis of differences in cross-attraction between the sexes of these species, differences in the egg weight in their reciprocal hybrid females, and the prevailing wind direction (Volney 1985). However, the patterns of gene flow between these species have not been explored. In Chapter 4, I use GBS to investigate the population structure and identify hybrids between *C. fumiferana*, *C. biennis* and *C. occidentalis* within a putative hybrid zone spanning much of central Alberta and central BC, south to the Canada-United States border. I then test multiple gene flow models to determine the extent and direction of gene flow between these three species.

In Chapter 5, I extend the analysis of the putative hybrid zone investigated in Chapter 4 to look at the locus-specific patterns of introgression and divergence between these three spruce budworm species. Of particular interest is the question of how the extent of phylogenetic divergence between species relates to genetic differentiation distributed across the genome. For instance, do the same loci significantly differentiate species pairs with recent versus deep phylogenetic histories? Likewise, do these loci experience similar forms of selection as

speciation proceeds? The answers to such questions are fundamental in understanding the genetic basis of reproductive isolation. Here, I integrate tests for selection with patterns of differential introgression across genomic and geographic clines between *C. fumiferana* and *C. occidentalis*, and *C. biennis* and *C. occidentalis,* to identify loci with putative roles in adaptation and maintaining species boundaries. Differences between *C. fumiferana* and *C. occidentalis*, and *C. biennis* and *C. occidentalis,* are investigated as examples of deep and recent divergences within the spruce budworm phylogeny, respectively (Sperling and Hickey 1994; Lumley and Sperling 2011a; Bird 2013). I then examine correspondences among the loci subject to selection, as well as the modes of selection in each comparison, to determine if similar genomic regions and evolutionary processes are implicated at successive stages of species divergence within the spruce budworm species complex, as has been demonstrated in other biological systems (Nadeau *et al*. 2013; Powell *et al*. 2013).

To fully understand the genetic architecture of reproductive barriers requires knowledge of the organization and linkage relationships between significantly differentiated regions of the genome. For instance, species boundaries may be maintained in small genomic regions linked to loci under selection or in regions distributed across the genome as a result of the coupling of endogenous and exogenous barriers to speciation (Michel *et al.* 2010; Via 2012; Powell *et al*. 2013; Bierne *et al*. 2014). However, the spruce budworm presently lacks a genetic linkage map to investigate such genomic characteristics. Accordingly, in Chapter 6 I employ GBS to develop a preliminary genetic linkage map for an outbred hybrid backcross family between *C. occidentalis* and *C. biennis*. Ultimately, this genetic resource will provide the means to investigate the role of genome structure in the maintainence of species boundaries within the spruce budworm species complex.

Thus, the overall aim of the work presented in this thesis is two-fold: i) to develop genetic and genomic resources that, in addition to providing a foundation for future work on the genomic architecture of species differences within the spruce budworm species complex, can also be used for practical purposes such as species identification; and ii) to obtain a clearer picture of the extent of hybrid interactions between some of the most destructive defoliators of North American conifers.

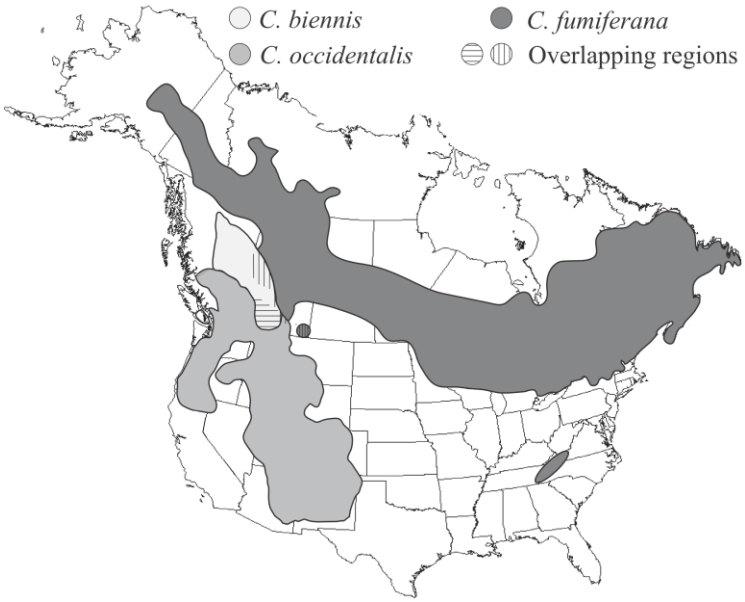


Figure 1-1. Species distributions of the spruce budworm, *Choristoneura fumiferana*, the western spruce budworm, *C. occidentalis*, and the two-year-cycle spruce budworm, *C. biennis*. Modified from Figure 4-3 in Lumley (2010).

Literature Cited

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Most, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice., A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., stelkens, R., Szymura, J.M., Vainola, R., Wolf, J.B.W., and D. Zinner. 2012. Hybridization and speciation. Journal of Evolutionary Biology, 26: 229-246.

Arnold, M.L., and N.H. Martin. 2009. Adaptation by introgression. Journal of Biology, 8: 82.

Bierne, N., Welch, J., Loire, E., Bonhomme, F., and P. David. 2011. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Molecular Ecology, 20: 2044-2072.

Bird, H.M. 2013. Phylogenomics of the *Choristoneura fumiferana* species complex (Lepidopter: Tortricidae). M.Sc. Thesis, University of Alberta, Edmonton, Canada.

Brunet, B.T.M., Hundsdoerfer, A.E., and F.A.H. Sperling. 2013. Identification and ecological characterisation of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada. The Canadian Entomologist, 145: 521-528.

Cadogan, B.L., Nealis, V.G., and K. van Frankenhuyzen. 1995. Control of spruce budworm (Lepidoptera: Tortricidae) with *Bacillus thuringiensis* applications times to conserve a larval parasitoid. Crop protection, 14: 31-36.

Campbell, I.M. 1958. A genetic study of factors determining fecundity in the genus *Choristoneura* Lederer (Clem.) (Tortricidae). PhD Thesis, University of Toronto, Toronto, Canada.

Campbell, I.M. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist 99: 482-486.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinuaer Associates, Inc., Sunderland, Massachusetts. Pp. 543.

Dang, P.T. 1985. Key to adult males of conifer-feeding species of *Choristoneura* (Lepidoptera: Tortricidae) in Canada and Alaska. The Canadian Entomologist, 117: 1-5.

Dasmahapatra, K.K., Walters, J.R., Briscoe, A.D., Davey, J.W., Whibley, A., Nadeau, N.J., Zimin, A.V., Hughes, D.S.T., Ferguson, L.C., Martin, S.H., Salazar, C., Lewis, J.J., Adler, S., Ahn, S.-J., Baker, D.A., Baxter, S.W., Chamberlain, N.L., Chauhan, R., Counterman, B.A., Dalmay, T., Gilbert, L.E., Gordon, K., Heckel, D.G., Hines, H.M., Hoff, K.J., Holland, P.W.H., Jacquin-Joly, E., Jiggins, F.M., Jones, R.T., Kapan, D.D., Kersey, P., Lamas, G., Lawson, D., Mapleson, D., Maroja, L.S., Martin, A., Moxon, S., Palmer, W.J., Papa, R., Papanicolaou, A., Pauchet, Y., Ray, D.A., Rosser, N., Salzberg, S.L., Supple, M.A., Surridge, A., Tenger-Trolander, A., Vogel, H., Wilkinson, P.A., Wilson, D., Yorke, J.A., Yuan, F., Balmuth, A.L., Eland, C., Gharbi, K., Thomson, M., Gibbs, R.A., Han, Y., Jayaseelan, J.C., Kovar, C., Mathew, T., Muzny, D.M., Ongeri, F., Pu, L.-L., Qu, J., Thornton, R.L., Worley, K.C., Wu, Y.-Q., Linares, M., Blaxter, M.L., ffrench Constant, R.H., Joron, M., Kronforst, M.R., Mullen, S.P., Reed, R.D., Scherer,

S.E., Richards, S., Mallet, J., McMillan, W.O., and C.D. Jiggins. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature, 487: 94-98.

Daterman, G.E., Sower, L.L., Stevens, R.E., and D.G. Fellin. 1995. Pheromone chemistry and response behaviour of conifer-feeding *Choristoneura* in the western United States. Pp. 69-84 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., and M.L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12: 499-510.

Davey, J.W., and M.L. Blaxter. 2010. RADSeq: next-generation population genetics. Briefings in Functional Genomics, 9: 416-423.

De Benedictis, J.A., Liebhold, A.M., and J.A. Powell. 1995. Studies of pheromone attraction and inheritance using virgin females of *Choristoneura* species, in the Central Sierra Nevada, California. Pp. 151-166 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Deverno, L.L., Smith,. G.A., and K.J. Harrison. 1998. Randomly amplified polymorphic DNA evidence of introgression in two closely related sympatric species of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae) in Atlantic Canada. Annals of the Entomological Society of America, 91: 248-259.

Dobesberger, E.J., Lim, K.P., and A.G. Raske. 1983. Spruce budworm (Lepidoptera: Tortricidae) moth flight from New Brunswick to Newfoundland. The Canadian Entomologist, 115: 1641-1645.

Ekblom, R., and J. Galindo. 2010. Applications of next generation sequencing in molecular ecology of non-model organsims. Heredity, 107: 1-15.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and S.E., Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One, 6(5): e19379. doi:10.10371/journal.pone.0019379.

Ennis, T.J. 1976. Sex chromatin and chromosome numbers in Lepidoptera. Canadian Journal of Genetics and Cytology, 18: 119-130.

Freeman, T.N. 1947. A new generic assignment for *Archips fumiferana* (Clemens), the spruce budworm (Lepidoptera, Tortricidae). The Canadian Entomologist, 79: 21-31.

Freeman, T.N. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. I. Some new forms of *Choristoneura* allied to *C. fumiferana*. The Canadian Entomologist, 99: 449-455.

Gardner, M.G., Fitch, A.J., Bertozzi, T., and A.J. Lowe. 2011. Rise of the machines - recommendations for ecologists when using next generation sequencing for microsatellite development. Molecular Ecology Resources, 11: 1093-1101.

Gray, T.G., Shepherd, R.F., and G.T. Harvey. 1995. Incidence of spicules on the aedeagi of *Choristoneura fumiferana, C. biennis*, and *C.*unidentified species (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 161-166.

Greenbank, D.O., Schaefer, G.W., and R.C. Rainey. 1980. Spruce budworm (Lepidoptera: Tortricidae) moth flight and dispersal: new understanding from canopy observations, radar, and aircraft. Memoirs of the Entomological Society of Canada, No. 110. pp. 49.

Harvey, G.T. 1957. The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). The Canadian Jounral of Zoology, 35: 549-572.

Harvey, G.T. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America V. Second diapause as a species character. The Canadian Entomologist, 99: 486-503.

Harvey, G.T. 1977. Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 109: 487-496.

Harvey, G.T. 1983a. A geographical cline in egg weights in *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and its significance in population dynamics. The Canadian Entomologist, 115: 1103-1108.

Harvey, G.T. 1983b. Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 115: 1109-1117.

Harvey, G.T. 1985a. The taxonomy of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): A review. Pp. 16-59 in C.J. Sanders, R.W. Stark, E.J. Mullins, And J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Harvey, G.T. 1985b. Egg weight as a factor in the overwintering survival of spruce budworm (Lepidoptera: Tortricidae) larvae. The Canadian Entomologist, 117: 1451-1461.Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferphagous *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519-536.

Hedrick, P.W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology, 22: 4606-4618.

Lawrence, R.K., Mattson, W.J., and R.A., Haack. 1997. White spruce and the spruce budworm: defining the phonological window of susceptibility. The Canadian Entomologist, 129: 291-318.

Liebhold, A.M. 1986. Larval coloration of hybrids between *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae). The Canadian Entomologist, 118: 857-860.

Liebhold, A.M., and W.J.A. Volney. 1984. Effect of temporal factors on reproductive isolation between *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae). The Canadian Entomologist, 116: 991-1005.

Liebhold, A.M., Volney, W.J.A., and W.E. Waters. 1984. Evaluation of cross-attraction between sympatric *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 827-840.

Lumley, L.M. 2010. Species delimitation in the *Choristoneura fumiferana* species complex (Lepidoptera: Tortricidae). Ph. D. Thesis. University of Alberta, Edmonton, Canada.

Lumley, L.M., and F.A.H. Sperling. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology, 35: 416-428.

Lumley, L.M., and F.A.H. Sperling. 2011a. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232-243.

Lumley, L.M., and F.A.H. Sperling. 2011b. Life-history traits maintin the genomic integrity of sympatric species of the spruce budworm (*Choristoneura fumiferana*) group on an isolated forest island). Ecology and Evolution, 1: 119-131.

Lysyk, T.J. 1989. Stochastic model of eastern spruce budworm (Lepidoptera: Tortricidae) phenology on white spruce and balsam fir. Journal of Economic Entomology, 82: 1161-1168.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution, 20: 229-237.

Martin, N.H., Bouck, A.C., and M.L. Arnold. 2006. Detecting adaptive trait introgression between Iris fulva and I. brevicaulis in highly selective field conditions. Genetics, 172: 2481-2489.

Matute, D.R., Butler, I.A., Turissini, D.A., and J.A. Coyne. 2010. A test of the snowball theory for the rate of evolution of hybrid incompatibilities. Science, 329: 1518-1521.

McGugan, B.M. 1954. Needle-mining habits and larval instars of the spruce budworm. The Canadian Entomologist, 86: 439-454.

Michel, A.P., Sim, S., Powell, T.H.Q., Taylor, M.S., Nosil, P., and J.L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences, 107: 9724-9729.

Miller, C.A. 1958. The measurement of spruce budworm populations and mortality during the first and second larval instars. The Canadian Jounral of Zoology, 36: 409-422.

Morjan, C.L., and L.H. Rieseberg. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Molecular Ecology, 13: 1341-1356.

Nadeau, N.J., Martin, S.H., Kozak, K.M., Salazar, C., Dasmahapatra, K.K., Davey, J.W., Baxter, S.W., Blaxter, M.L., Mallet, J., and C.D. Jiggins. 2013. Genome-wide patterns of divergence and gene flow across a butterfly radiation. Molecular Ecology, 22: 814-826.

Nealis, V.G. 2005. Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist, 137: 584-597.

Nealis, V.G. 2008. Spruce budworms, *Choristoneura* Lederer (Lepidoptera: Tortricidae). Pp. 3524-3531 in J.L. Capinera (Ed.), Encyclopedia of Entomology, 2nd edition. Springer, Dordrecht. Pp. 4411.

Nealis, V.G., and J. Regniere. 2009. Risk of dispersal in western spruce budworm. Agricultural and Forest Entomology, 11: 213-223.

Nolte, A.W., Gompert, Z., and C.A. Buerkle. 2009. Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. Molecular Ecology, 18: 2615-2627.

Nosil, P., Vines, T.H., and D.J. Funk. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution, 59: 705-719.

Nosil, P. 2012. Ecological Speciation. Oxford University Press, University of Oxford, Oxford, United Kindom. 280pp.

Orr, H.A., and M. Turelli. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution, 55: 1085-1094.

Parsons, T.J., Olson, S.L., and M.J. Braun. 2006. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. Science, 260: 1643-1646.

Poland, J.A., Brown, P.J., Sorrells, M.E., and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLOS One 7:e32253. doi:10.1371/journal.pone.0032253.

Powell, J.A., and J.A. DeBenedictis. 1995. Evolutionary interpretations, taxonomy, and nomenclature. Pp. 217-275 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Powell, T.H.Q., Hood, G.R., Murphy, M.O., Heilveil, J.S., Berlocher, S.H., Nosil, P., and J.L., Feder. 2013. Genetic divergence along the speciation continuum: the transition from host race to species in Rhagoletis (Diptera: Tephritidae). Evolution, 67: 2561-2576.

Regniere, J., and R.M. Fletcher. 1983. Direct measurement of spruce budworm (Lepidoptera: Tortricidae) larval dispersal in forest stands. Environmental Entomology, 12: 1532-1538.

Rieseberg, L.H., Atrcher, M.A., and R.K. Wayne. Transgressive segregation, adaptation and speciation. Heredity, 83: 363-372.

Rowe, H.C., Renaut, S., and A. Guggisberg. 2011. RAD in the realm of next-generation sequencing technologies. Molecular Ecology, 20: 3499-3502.

Rundle, H.D., and P. Nosil. Ecological speciation. Ecology letters, 8: 336-352.

Sanders, C.J. 1971b. Sex pheromone specificity and taxonomy of budworm moths (*Choristoneura*). Science, 171: 911-913.

Sanders, C.J. 1971a. Daily activity patterns and sex pheromone specificty as sexual isolating mechanisms in two species of *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 103: 498-502.

Sanders, C.J., Daterman, G.E., and T.J. Ennis. 1977. Sex pheromone responses of *Choristoneura* spp. and their hybrids (Lepidoptera: Tortricidae). The Canadian entomologist, 109: 1203-1220.

Sanders, C.J. 1991. Biology of North American spruce budworm. Pp. 579–620, in L.P.S. van der Gesst and H.H. Evenhuis (Eds.), Tortricid pests, their biology, natural enemies and control. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Scascitelli, M., Whitney, K.D., Randell, R.A., King, M., Buerkle, C.A., and L.H. Rieseberg. 2010. Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H.*

debilis) reveals asymmetric patterns of introgression and small islands of genomic differentiation. Molecular Ecology, 19: 521-541.

Schmidt, F.H. and W.L. Lauer. 1977. Developmental polymorphism in *Choristoneura* spp. (Lepidoptera: Tortricidae). Annals of the Entomological Society of America, 70: 112-118.

Schmidt, F.H., Campbell, R.K., and S.J. Trotter, Jr. 1977. Errors in determining instar numbers through head capsule measurements of a lepidopteran - a laboratory study and critique. Annals of the Entomological Society of America, 70: 750-756.

Shepherd, R.F., Gray, T.G., and G.T. Harvey. 1995. Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies, Picea,* and *Pseudotsuga* in western Canada and Alaska. The Canadian Entomologist, 127: 813-830.

Smith, S.G. 1953. Reproductive isolation and the integrity of two sympatric species of *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 85: 141-151.

Smith, S.G. 1954. A partial breakdown of temporal and ecological isolation between *Choristoneura* species (Lepidoptera: Tortricidae). Evolution, 8: 206-224.

Sperling, F.A.H., and D.A. Hickey. 1994. Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). Molecular Biology and Evolution, 11: 656-665.

Sperling, F.A.H., and D.A. Hickey. 1995. Amplified mitochondrial DNA as a diagnostic marker for species of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 277-288.

Stehr, G.W. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. II. Geographic distribution in accordance with forest regions. The Canadian Entomologist, 99: 456-463.

Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B, 367: 451-460.

Volney, W.J.A. 1985. Comparative population biologies of North American spruce budworms. Pp. 71-84 in C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Volney, W.J.A. and H.F. Cerezke. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. Canadian Jounral of Forest Research, 22: 198-205.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characterisitics. Global Change Biology, 13: 1630-1643.

Volney, W.J.A., Liebhold, A.M., and W.E. Waters. 1984. Host associations, phenotypic variation, and mating compatibility of *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 813-826.

Volney, W.J.A., and A.M. Liebhold. 1985. Post-diapause development of sympatric *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) and their hybrids. The Canadian Entomologist, 117: 1479-1488.

Volney, W.J.A, Waters, W.E., Akers, R.P., and A.M. Liebhold. 1983. Variation in spring emergence patterns among western *Choristoneura* spp. (Lepidoptera: Tortricidae) populations in southern Oregon. The Canadian Entomologist, 115: 199-209.

Wang, R.J., Ane, C., and B.A. Payseur. 2013. The evolution of hybrid inompatibilities along a phylogeny. Evolution, 67: 2905-2922.

Zane, L., Bargelloni, L., and T. Patarnello. 2002. Strategies for microsatellite isolation: a review. Molecular Ecology, 11: 1-16.

Chapter 2

Identification and ecological characterization of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada

Summary

The western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), is an important pest of western North American coniferous forests, where its principal larval host is Rocky Mountain Douglas-fir, *Pseudotsuga menziesii* subspecies *glauca* (Beissner) Murrary (Pinaceae). In southwestern Alberta, Canada, populations were recently discovered feeding on an unconventional host, an Engelmann spruce, *Picea engelmannii* Parry ex. Engelmann X white spruce, *Picea glauca* (Moench) Voss hybrid (Pinaceae), in a transition zone between the two major forest types characterized by these conifer species. We use molecular evidence to verify the species identity of outbreaking *Choristoneura* populations collected from Douglas-fir and hybrid spruces in southwestern Alberta in 2009, and characterize the larval and male moth phenology. Both mitochondrial and microsatellite markers confirmed these populations as *C. occidentalis*, but admixture with *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) was detected in some individuals. Few differences associated with separate host trees were evident in the phenology of larvae and the flight period of male moths.

Introduction

The Western Spruce Budworm (WSBW), *Choristoneura occidentalis* (Freeman) (Lepidoptera: Tortricidae), has become a forest pest species of concern in Alberta, Canada, due

to its apparently recent presence within the province and its rapid population increase since initial detection. Defoliation of stands dominated by Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco subsp. *glauca* (Beissner) A.E. Murray (Pinaceae), in the Porcupine Hills of southwestern Alberta was first documented in 2005 and the defoliator responsible was provisionally identified as WSBW (Alberta Environment and Sustainable Resource Development 2005). By 2008, over 20 000 hectares of forest in and around the Porcupine Hills had moderate levels of defoliation (Alberta Environment and Sustainable Resource Development 2009). Populations of WSBW have also become established in neighbouring low elevation regions of Alberta where white spruce, *Picea glauca* (Moench) Voss (Pinaceae), and a recognised hybrid spruce, *Picea englemannii* Parry ex. Engelmann (Pinaceae) x *P. glauca*, are dominant conifers (Strong and Hills 2006). Strong and Hills (2006) referred to this hybrid as *Picea albertiana* S. Brown subsp. *ogilviei* Strong & Hills. However, it is unknown whether WSBW develops differently on its alternate hosts in this area, or whether hybrid spruce facilitates WSBW movement between Douglas-fir and non-Douglas-fir dominant forests.

The range of WSBW is largely congruent across western North America with that of Douglas-fir, its primary host (Stehr 1967; Harvey 1985). In Alberta, the Porcupine Hills have the most extensive Douglas-fir stands in the province and are the northeastern limit of the historical range of WSBW (Harvey 1985; Shepherd *et al.* 1995). However, southwestern Alberta is a putative hybrid zone between several budworm species (Shepherd *et al*. 1995; Lumley and Sperling 2011a), which puts the species identity of the outbreaking populations into question.

At least eight species comprise the spruce budworm (SBW), *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) species complex (Harvey 1985; Volney and Fleming 2007; Lumley and Sperling 2010), and their ranges overlap in transitional regions between major forest

types characterised by their coniferous hosts (Stehr 1967; Volney and Fleming 2007). In Alberta, the range of SBW extends across the northern boreal forest, where white spruce is dominant, and southward along the foothills where it contacts WSBW (Lumley and Sperling 2011a). Isolated populations of these two species also occur in the Cypress Hills, on the boundary between Alberta and Saskatchewan (Lumley and Sperling 2011b). A third species, the two-year cycle budworm, *Choristoneura biennis* Freeman (Lepidoptera: Tortricidae), feeds on Engelmann spruce and subalpine fir, *Abies lasiocarpa* (Hooker) Nuttall (Pinaceae). It contacts the ranges of both SBW and WSBW (Stehr 1967), but is separated from them by the occurrence of its primary hosts at higher elevations and its obligatory second diapause during the fourth larval instar (Harvey 1967; Nealis 2005). At least two pine-feeding species, *Choristoneura pinus* Freeman and *Choristoneura lambertiana* (Busck) (Lepidoptera: Tortricidae) also occur in parts of central and southern Alberta.

Since spruce budworm species display significant phenological synchrony with their hosts (Volney and Cerezke 1992), which is reinforced by selection on extensive variation in the timing of post-diapause larval emergence of overwintered second instar larvae (Volney *et al*. 1983; Thomson *et al*. 1984; Shepherd 1985), developmental differences can be expected to result from any establishment on alternative hosts with different phenological schedules. This may be more pronounced in areas with hybrid populations, where selection on such traits can be elevated relative to pure populations (Volney *et al*. 1983). In our study, we compare the timing of the development on Douglas-fir and hybrid spruce by *Choristoneura* populations responsible for the recent outbreak in southwestern Alberta, and we use a combination of molecular techniques to determine their species identity.

Methods

Study sites and sampling design

Choristoneura larvae, pupae, and adults were sampled at five locations in the Crowsnest Pass region of southwestern Alberta (Figure 2-1). Two 45 cm branch tips were pruned from the mid-crown of a single Douglas-fir or hybrid spruce tree at each location, with sampling occurring between 20 May and 8 August 2009 at 8–10 day intervals. Species identity of the trees was determined using seed cone morphology (Farrar 1995; Strong and Hills 2006). All larval instars were collected into 95% ethanol and were delimited by measuring head capsule width to the nearest 10 μm and developing instar separation criteria using the program HCAP (Logan *et al.* 1998). Pupae were collected live and reared to eclosion. Average observed instar ($AI_{obs} = \sum p_{ii}$ where p_i is the proportion of WSBW in each instar i (Lysyk 1988)) was calculated for each site and tree species and sampling date. Adult flight period was monitored between 12 July and 30 September 2009 by deploying two Multi-Pher I insect traps (Services Bio-Contrôle, Ste-Foy, Québec, Canada) with eastern spruce budworm flex lures (Contech, Victoria, British Columbia, Canada) and Vaportape II™ (Hercon, Emigsville, Pennsylvania, United States of America) at each site. Cumulative proportion of male moth trap catch throughout the flight period was arcsine square-root transformed to approach normality. The effects of tree species and site on the relationship between AI_{obs} and day of year, and transformed cumulative proportion of male moths and day of year, were tested using general linear models testing the homogeneity of slopes, *e.g.*, AI_{obs} = day of year + site + day of year*site in R 2.15.1 (R Core Team 2012).

Budworm species identity

To verify the species identity of budworms at each site, we used two types of molecular markers: mitochondrial (mt) DNA and microsatellites. We sequenced a 470-base pair region of the cytochrome oxidase I (*coI)* mt gene in 41 adults (8–9 specimens per location) using the methods of Sperling and Hickey (1994) and Lumley and Sperling (2010). Four adults collected at pheromone traps and 4–5 reared from larvae were used. Their *coI* sequences were then related to 169 previously published *coI* haplotypes (Sperling and Hickey 1994; Roe and Sperling 2007; Lumley and Sperling 2010, 2011a, 2011b), using maximum parsimony and likelihood as in Lumley and Sperling (2011a).

For microsatellites, we genotyped each of the 41 individuals at eight previously published loci (Lumley *et al*. 2009) and analysed the data as in Lumley and Sperling (2011a, 2011b) using *structure* v2.3.2 (Pritchard *et al.* 2000). Data for the 1135 specimens of Lumley and Sperling (2011a), encompassing all known North American spruce budworm species, was used to train the admixture model with locations. Genotypes for the 41 new southwestern Alberta specimens were then used to infer assignment probabilities for each of six previously defined populations, corresponding to *C. fumiferana*, Western A, Western B, *C. lambertiana*, *C. pinus*, and *C. retiniana* genetic clusters (Lumley and Sperling 2011a).

Results

A total of 698 larvae, 149 pupae, and 7189 male moths were collected over 132 days (18 sampling times) (Table 2-1). Larval abundance was highest at Beaver Mines and lowest at Porcupine South, Alberta with 239 and 104 larvae/pupae collected in total from each site, respectively (Table 2-1). The average observed instar (AI_{obs}) at each sampling time and site is

shown in Figure 2-2 along with the cumulative proportion of male moths throughout the flight period at each location. The relationship between AI_{obs} and ordinal date did not differ significantly among sites ($F_{4,32} = 0.2856$; $P = 0.8851$) or hosts ($F_{1,38} = 1.223$; $P = 0.2758$).

Male moth abundance was highest at Coleman (2553 moths) and lowest at Porcupine South (870 moths) (Table 2-1). However, when abundance is represented as the cumulative proportion of moths relative to the total caught per site to account for potential differences in population size (Figure 2-2), there are no significant differences in the relationship between male moth flight phenology and ordinal date between sites ($F_{4,35} = 0.3981$; $P = 0.8086$) or hosts ($F_{1,41} = 0.3533$; $P = 0.5555$).

Species identity

Maximum parsimony and likelihood analyses of new mtDNA sequences combined with previously published haplotypes gave trees with very similar topologies and the same major lineages as in previous studies (Sperling and Hickey 1994; Lumley and Sperling 2010, 2011a, 2011b). Thirty-nine *o*-lineage and two *oβ*-lineage *coI* haplotypes were found among the 41 specimens from southwestern Alberta (Table 2-2). Three new haplotypes (o94, o95, and o96; GenBank accession numbers KC172836:KC172838) differed by at least one base pair from known haplotypes. No *f*-lineage haplotypes, characteristic of *C. fumiferana,* were sampled at the five locations.

Microsatellite analyses were also congruent with previous work (Lumley and Sperling 2011a, 2011b). Each of the 41 specimens from southwestern Alberta was assigned to the previously known genetic clusters (Table 2-2). Most were assigned to Population 2 (Western A,

n = 26) or Population 3 (Western B, *n* = 12) genetic clusters, with approximately equal numbers of reared and pheromone-trapped specimens included in each cluster. The three remaining individuals were assigned to Population 1 (*C. fumiferana*, *n* = 3), with two from Coleman (1 reared on Douglas-fir and 1 pheromone-trapped) and one from Maycroft (pheromone-trapped). However, each of these three individuals had *o*-lineage mtDNA, and assignment probabilities to the Population 1 microsatellite cluster were not high (44%, 61%, and 72%). Although the Western A and B genetic clusters defined by Lumley and Sperling (2011a) contained species in addition to *C. occidentalis* that are potentially present in southwestern Alberta, none of our larval specimens exhibited the second diapause characteristic of *C. biennis*, nor were any larvae taken from pines or adults from the pheromone lures normally associated with *C. lambertiana* (Harvey 1985).

Discussion

DNA evidence as well as life history traits (lack of second diapause and type of pheromone attraction), indicate that the *Choristoneura* populations responsible for severe defoliation of coniferous forests in southwestern Alberta are best identified as WSBW. Although the presence of this species in Alberta is not new (Harvey 1985; Shepherd *et al.* 1995), it does represent the first major outbreak of WSBW in the province (Sustainable Resource Development 2007). Aerial surveys conducted concurrently with the work described in this study estimated more than 30 000 ha of forest with moderate to severe levels of defoliation in the Porcupine Hills and surrounding areas (Sustainable Resource Development 2010). This outbreak declined

precipitously to just 2900 ha in 2010 (Sustainable Resource Development 2011), but larval populations remain high in restricted areas on Douglas-fir and hybrid spruce.

Our study is also the first report of WSBW feeding freely as larvae on an unconventional conifer host, hybrid spruce, within the province. However, feeding on this alternative host to Douglas-fir had no apparent impact on rate of development. We failed to find any significant effect of site or host on the relationship between AI_{obs} and ordinal date. Second instar larvae were first observed in late May (day 140). Late instar larvae and pupae were found up to the date of the last larval collection at the beginning of August (day 220). Similarly, the cumulative proportion of male moths caught throughout the sampling period did not differ between sites or hosts. Male moths were first observed on 21 July (day 201), but may have been present as early as 12 July (day 193) when the pheromone traps were first deployed and pupae were first observed. Peak densities of male moths were observed between 17–26 August (days 229–238) and moths were still present in low densities at four of the five sites at the end of September (day 273).

The use of hybrid spruce as a larval host by WSBW is not unexpected, since the patchy landscape of the cordilleran region of western North America may predispose WSBW populations to tolerate variation in host-tree species (Volney 1985). In fact, WSBW are known to feed on firs, Douglas-fir and spruces (Fellin and Dewey 1982; Sanders 1991), in addition to one other white spruce x Engelmann spruce hybrid, which has long been identified as a host for budworms located in the hybrid zone between white spruce and Engelmann spruce (Stehr 1967). However, oviposition preferences by gravid females suggest that firs and Douglas-fir are the favoured hosts (Leyva *et al*. 2000, 2003).

The relatively labile feeding behavior of WSBW raises the possibility that hybrid stands may facilitate budworm movement between stands of more favourable hosts. The "hybrid-bridge" hypothesis, for instance, proposes that hybrid host trees may act as physiological intermediates between parental plant species and allow sustained host-shifts by herbivores (Floate and Whitman 1993). A current example is the expansion of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), onto Jack pine, *Pinus banksiana* Lambert (Pinaceae), in Alberta via hybrids between Jack pine and the closely related lodgepole pine, *Pinus contorta* Douglas (Pinaceae) (Cullingham *et al.* 2011). Although there is no evidence that WSBW is experiencing a similar host shift in southwest Alberta, it is noteworthy that its populations are surviving on hybrid spruces that not only bridge the region between boreal and montane forests along the eastern slopes of the Rocky Mountains, but also range far into Canada's interior (Strong and Hills 2006).

We also found evidence for hybridisation among WSBW and SBW in the populations surveyed. Although all 41 individuals analysed for microsatellite characteristics had some degree of admixture between the six pre-defined genetic clusters, three individuals were not assigned to genetic clusters associated with WSBW. Instead these three individuals were assigned to the genetic cluster associated with SBW. All individuals also had *o*- or *oβ*- lineage haplotypes, indicating that hybridisation between these two species would have resulted from matings between male SBW and female WSBW. These results are highly concordant with recent reports of genetic introgression among budworm species whose ranges overlap in Alberta (Lumley and Sperling 2011a, 2011b). They are also supported by mating experiments that suggest introgression between WSBW and SBW may be most likely to occur via a male SBW, since egg weights from that combination are greater than the reciprocal cross (Harvey 1997). Previous

studies have shown that hybrid populations can rapidly adapt to divergent developmental patterns of different host species (Volney *et al*. 1983; Volney 1999); however the survival rates of hybrids have not been examined for southwestern Alberta.

In summary, WSBW is successfully establishing itself at the edge of its range in southwestern Alberta. Populations on Douglas-fir and hybrid spruce in the Porcupine Hills and surrounding areas do not differ developmentally, at least at the scale of this study. Fine-scaled phenology models and within-site comparisons would shed light on the developmental consequences of different host associations, and should eventually benefit the management of these populations. Moreover, investigation of the genetic structure of the hybrid zone between WSBW and SBW, and any correlations with hybridisation between white spruce and Engelmann spruce, may provide useful insight into the evolutionary and ecological characteristics of the spruce budworm species complex.

Table 2-1: Sample size of larval/pupal and adult (male) *Choristoneura* collections by ordinal date. Parentheses denote adult collections that occurred concurrently with larval collections.

Day	Beaver Mines	Coleman	Maycroft	Porcupine North	Porcupine South
140/ 141	0	2	0	0	0
148	14	9	9	0	6
157	10	14	33	0	1
166	78	17	19	15	12
175	47	12	30	24	12
184	23	19	51	24	16
193	29	21	25	20	19
201/ 202	22 (42)	31 (107)	28 (29)	25 (12)	18 (9)
211	16 (115)	17 (249)	26 (100)	14 (116)	14 (63)
220	350	5 (548)	8 (248)	6 (193)	6 (105)
229	478	624	261	161	253
238	351	845	265	447	345
246	46	103	53	371	79
255	13	47	6	53	4
264	5	11	27	10	8
273	0	9	23	1	4
Immatures	239	147	229	128	104
Adults	1400	2543	1012	1364	870
Total	1639	2690	1241	1492	974

Table 2-2: *Choristoneura* collection site, method of collection (adult reared from larva or pheromone trapped), and combined genetic profile (microsatellite population /mtDNA *co1* haplotype (sample size)). Previously named haplotypes are as in Sperling and Hickey (1994) and Lumley and Sperling (2010, 2011a, 2011b).

Site	Collection Method	Combined Genetic Profile
Beaver Mines	Reared	2/b1(1), 2/o2(3), 3/o2(1)
Beaver Mines	Trapped	2/b1(1), 2/o5(1), 3/o1(1), 3/o2(1)
Coleman	Reared	1/b1(1), 2/b1(1), 2/o2(1), 2/o3(1),
Coleman	Trapped	1/o2(1), 2/oß(1), 3/o2(2)
Maycroft	Reared	2/o1(1), 2/o2(1), 2/o94(1)*, 3/o11(1)
Maycroft	Trapped	1/o2(1), 2/o2(2), 2/o28(1)
Porcupine North	Reared	2/o2(1), 2/o95(1)*, 3/o2(2)
Porcupine North	Trapped	2/o1(1), 2/o5(1), 2/o41(1), 3/o11(1)
Porcupine South	Reared	2/oß(1), 2/b1(1), 3/o2(2)
Porcupine South	Trapped	2/o1(1), 2/o2(1), 2/o28(1), 3/o96(1)*

*New mtDNA *co1* haplotypes, Genbank accession numbers KC172836:KC172838

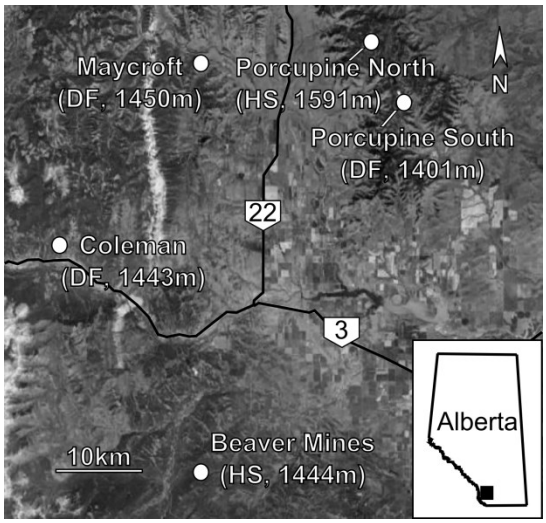


Figure 2-1. Southwestern Alberta, Canada sample sites showing larval hosts (hybrid spruce, Douglas-fir), and elevation. Photograph is from Google Maps (https://maps.google.com), with the addition of major highways and location of the study areas within Alberta.

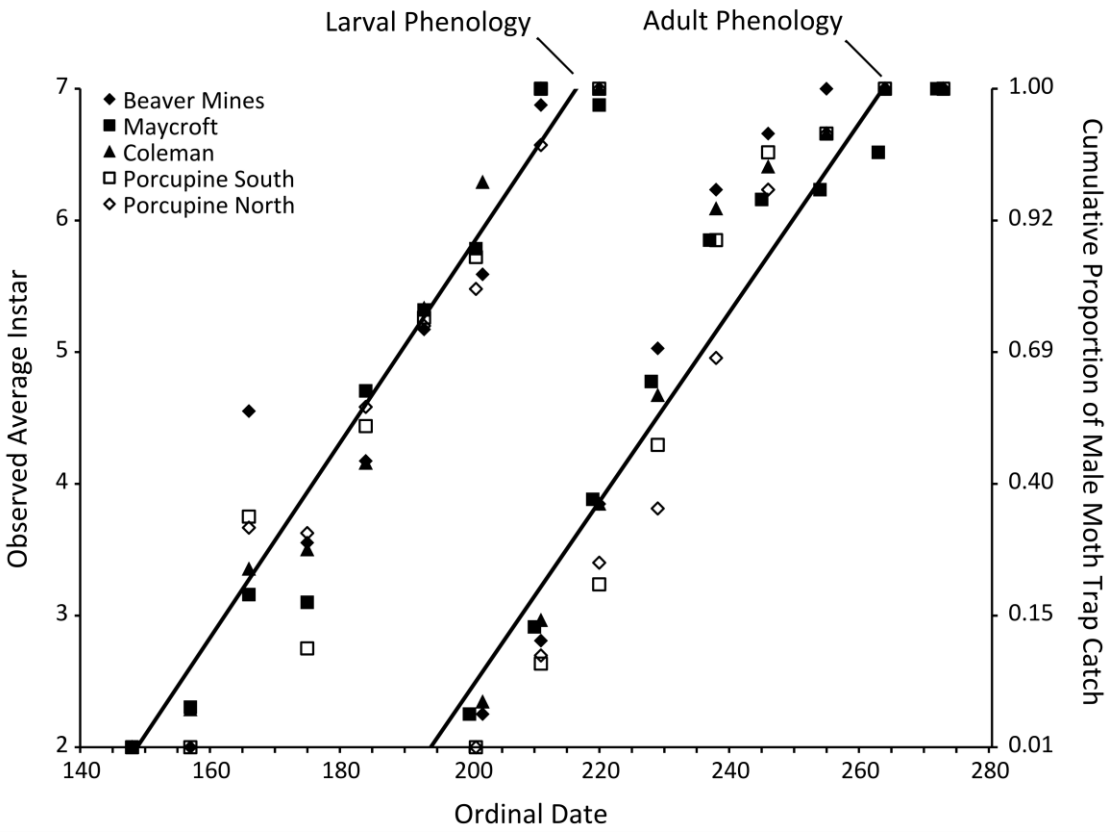


Figure 2-2. Developmental progression of *Choristoneura* populations at five sites in southwestern Alberta, Canada. Left side shows observed average instar for each site and the regression line for all sites combined, with two to six for larval instars and seven for pupae. Right side show arcsine square-root transformed cumulative proportion of male moth abundance per collection date at each set with the regression line for all sites combined. The right axis is presented back-transformed. Horizontal axis shows ordinal date.

Literature Cited

Alberta Environment and Sustainable Resource Development. 2005. Forest Health in Alberta, 2005 Annual Report. Edmonton, Alberta, Pp. 22.

Alberta Environment and Sustainable Resource Development. 2007. Forest Health in Alberta, 2006 Annual Report. Edmonton, Alberta, Pp. 58.

Alberta Environment and Sustainable Resource Development. 2009. Forest Health in Alberta, 2008 Annual Report. Edmonton, Alberta, Pp. 50.

Alberta Environment and Sustainable Resource Development. 2010. Forest Health in Alberta, 2009 Annual Report. Edmonton, Alberta, Pp. 48.

Alberta Environment and Sustainable Resource Development. 2011. Forest Health in Alberta, 2010 Annual Report. Edmonton, Alberta, Pp. 54.

Cullingham, C.I., Cooke, J.E.K., Dang, S., Davis, C.S., Cooke, B.J., and D.W. Coltman. 2011. Mountain pine beetle host-range expansion threatens the boreal forest. Molecular Ecology, 20: 2157–2171.

Farrar, J.L. 1995. Trees in Canada. Natural Resources Canada, Canadian Forest Service, Ottawa, Ontario, Canada and Fitzhenry and Whiteside Limited, Markham, Ontario, Canada.

Fellin, D.G., and J.E. Dewey. 1982. Western spruce budworm. Forest Insect & Disease Leaflet 53, United States Department of Agriculture, Forest Service, Missoula, Montana, United States of America.

Floate, K.D., and T.G. Whitham. 1993. The "Hybrid Bridge" hypothesis: host shifting via plant hybrid swarms. The American Naturalist, 141: 651–662.

Harvey, G.T. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. V. Second diapause as a species character. The Canadian Entomologist, 99: 486–503.

Harvey, G.T. 1985. The taxonomy of the coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): a review. *In* Recent advances in spruce budworms research. Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September, 1984. *Edited by* C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy. Canadian Forest Service, Ottawa, Ontario, Canada. Pp. 16–48.

Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferophagous *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519–536.

Leyva, K.J., Clancy, K.M., and P.W. Price. 2000. Oviposition preference and larval performance of the western spruce budworm (Lepidoptera: Tortricidae). Environmental Entomology, 29: 281–289.

Leyva, K.J., Clancy, K.M., and P.W. Price. 2003. Oviposition strategies employed by the western spruce budworm: tests of predictions from the phylogenetic constraints hypothesis. Agricultural and Forest Entomology, 5: 9–16.

Logan, J.A., Bentz, B.J., Vandygriff, J.C., and D.L. Turner. 1998. General program for determining instar distributions from widths: example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. Environmental Entomology, 27: 555–563.

Lumley, L.M., Davis, C.S., and F.A.H. Sperling. 2009. Isolation and characterization of eight microsatellite loci in the spruce budworm species *Choristoneura fumiferana* and *Choristoneura occidentalis*, and cross-species amplification in related tortricid moths. Conservation Genetic Resources, 1: 501–504.

Lumley, L.M., and F.A.H. Sperling. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology, 35: 416–428.

Lumley, L.M.L., and F.A.H. Sperling. 2011a. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232–243.

Lumley, L.M.L., and F.A.H. Sperling. 2011b. Life-history traits maintain the genomic integrity of sympatric species of the spruce budworm (*Choristoneura fumiferana*) group on an isolated forest island. Ecology and Evolution, 1: 119–131.

Lysyk, T.J. 1988. A multiple-cohort model for simulating jack pine budworm (Lepidoptera: Tortricidae) development under variable temperature conditions. The Canadian Entomologist, 121: 373–387.

Nealis, V.G. 2005 Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist, 137: 584–597.

Pritchard, J.K., Stephens, M., and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Roe, A.D., and F.A.H. Sperling. 2007. Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. Molecular Ecology, 16: 3617–3633.

Sanders, C.J. 1991. Biology of North American spruce budworm. *In* Tortricid pests, their biology, natural enemies and control. *Edited by* L.P.S. van der Gesst and H.H. Evenhuis. Elsevier Science Publishers B.V., Amsterdam, Netherlands. Pp. 579–620.

Shepherd, R.F. 1985. A theory on the effects of diverse host-climatic environments in British Columbia on the dynamics of western spruce budworm. *In* Recent advances in spruce budworms research. Proceedings of the CANUSA spruce budworms research symposium, Bangor, Maine, 16-20 September, 1984. *Edited by* C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy. Canadian Forestry Service, Ottawa, Ontario, Canada. Pp. 60–70.

Shepherd, R.F., Gray, T.G., and G.T. Harvey. 1995. Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies*, *Picea*, and *Pseudotsuga* in western Canada and Alaska. The Canadian Entomologist, 127: 813–830.

Sperling, F.A.H., and D.A. Hickey. 1994. Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). Molecular Biology and Evolution, 11: 656–665.

Stehr, G.W. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America, II. Geographic distribution in accordance with forest regions. The Canadian Entomologist, 99: 456-463.

Strong, W.L., and L.V. Hills. 2006. Taxonomy and origin of present-day morphometric variation in *Picea glauca* (x *engelmannii*) seed-cone scales in North America. Canadian Journal of Botany, 84: 1129–1141.

Thomson, A.J., Shepherd, R.F., Harris, J.W.E., and R.H. Silversides. 1984. Relating weather to outbreaks of western spruce budworm, *Choristoneura occidentalis* (Lepidoptera: Tortricidae), in British Columbia. The Canadian Entomologist, 116: 375–381.

Volney, W.J.A. 1999. Genetic and ecological controls of post-diapause development in *Choristoneura*. *In* Physiology and genetics of tree-phytophage interactions, Gujan, France, August 31 to September 5, 1997. *Edited by* F. Lieutier, W.J. Mattson, and M.R. Wagner. Institut National de la Recherche Agronomique (INRA), Paris, France. Pp. 187–195.

Volney, W.J.A., and H.F. Cerezke. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. Canadian Journal of Forest Research, 22: 198–205.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characteristics. Global Change Biology, 13: 1630–1643.

Volney, W.J.A., Waters, W.E., Akers, R.P., and A.M. Liebhold. 1983. Variation in spring emergence patterns among western *Choristoneura* spp. (Lepidoptera: Tortricidae) populations in southern Oregon. The Canadian Entomologist, 115: 119–209.

Chapter 3

Characterization of EST-based SSR loci in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae)

Summary

Choristoneura fumiferana expressed sequence tags were used to discover 114 microsatellite loci *in silico*. 87 of these loci were assayed in a panel of 11 wild-caught individuals resulting in 29 polymorphic loci. Further analysis of 20 of these loci in 31 individuals collected from a single wild population in northern Minnesota identified 14 loci in Hardy-Weinberg equilibrium.

Main Text

Expressed sequence tags (ESTs) are valuable resources for rapid discovery of genetic variation, including microsatellites (simple sequence repeats, SSRs). As their name implies, ESTs are derived from transcribed (exonic and untranslated) regions of the genome and are therefore relatively conserved compared to neutral regions. Thus, SSRs occurring in ESTs (EST-SSR), and the sequences flanking these markers, generally have overall lower levels of polymorphism (Kim *et al*. 2008). Consequently, EST-SSRs typically have a lower incidence of null alleles, as has been shown in other systems (Kim *et al.* 2008). This is particularly beneficial for SSR development in Lepidoptera, insects known to have high levels null alleles, as well as, transposable elements that can complicate marker development (Sinama *et al.* 2011; van't Hof *et al*. 2007; Zhang 2004).

The spruce budworm [*Choristoneura fumiferana* (Clemens)] is the most ecologically important defoliator of North American coniferous forests (Volney and Fleming 2007). Although the spruce budworm is not itself an endangered species, it is nonetheless of great conservation

interest because of its role in determining structure within North America's boreal forest ecozone. Its geographical distribution covers the boreal forest across Canada ranging from the Atlantic coast to the Yukon and Alaska, an area of ~1 billion hectares of forested land (Volney and Fleming 2000). As one of the world's largest resources of sequestered carbon (and therefore a significant contributor to the global carbon cycle), the boreal forest is of global conservation importance. Ironically however, the boreal forest is increasingly threatened by industry (*e.g.* oil exploration and extraction, timber harvesting) and natural disturbances (*e.g.* climate change, wildfire, insect outbreak). Impacts caused by the spruce budworm alone have been known to account for more than 30% of the total forested area depleted annually in Canada (Volney and Fleming 2000). Thus, the conservation of boreal ecozones would benefit from informed management of spruce budworm populations, particularly during outbreak phases.

Although spruce budworm population cycles over the last 400 years have been reconstructed using dendrochronological records (Boulanger *et al.* 2012), surprisingly little is known about the synchronization of population cycles and dispersal patterns of the spruce budworm throughout its range (Anderson and Sturtevant 2011). This gap is largely due to a paucity of genetic markers from which fine-scaled surveys of population structure can be attained. Here, we characterize SSRs derived from EST libraries of the spruce budworm that complement an existing SSR resource developed by Lumley *et al*. (2009) using traditional genomic isolation techniques.

Trace files for 41,230 ESTs of *C. fumiferana*, were base-called, assigned quality values, and trimmed of low-quality sequence using Phred v.0.071220.b (Ewing *et al*. 2008). Sequencing artifacts were removed from the quality filtered ESTs using Figaro v.1.05 (White *et al*. 2008) and SeqClean (http://compbio.dfci.harvard.edu/tgi/software/). Since the ESTs were sequenced only

from the 5' end of cDNA clones, TGICL (Pertea *et al.* 2002) was used to assemble the ESTs without considering reads in the reverse orientation. Approximately 1893 unigenes and 1406 singletons resulted from this processing.

Using a custom Perl script (Beldade *et al*. 2009) 150 EST-SSRs were discovered with at least three repeats of 2-5 bases in length. These were reduced to 114 unique loci after accounting for redundancy among loci using MicroFamily v.1.2 (Meglecz 2007). Among these, 63 SSRs were unique among all loci, and two sets of loci formed unique compound SSRs separated by short-stretches of non-repetitive sequence. All other loci were found to have redundancy within their flanking sequences with at least one other locus. Included among this redundant set of loci were those in which flanking sequences form reverse complements ("complement" in the unique column of Appendix 3-1). As these ESTs were derived from unidirecitional libraries, as noted above, these complementary sequences must represent paralogous genomic regions. Most unique loci had trinucleotide repeat motifs (58 loci) as would be expected to maintain consistency in the reading frame, and in general longer motifs were less frequent. Primer3 (Rozen and Skaletsky 2000) was used to find primer sequences between 20-25 bases in length that yielded amplicons between 100-150 bases for each locus. Primers for 87 loci were designed using these criteria (Appendix 3-1).

A validation panel of 11 *C. fumiferana* individuals (four females and seven males) collected throughout Alberta was used to test loci for amplification success. Microsatellites were amplified in 10 µL reactions containing 1X PCR buffer (10 mM Tris pH8.8, 0.1% Triton X-100, 50 mM KCl, 0.16 mg/ml BSA), 2.5 mM MgCl2, 0.2 mM dNTPs, 0.1 uM M13-tagged forward primer, 0.4 um reverse primer, 0.4 µm fluorophore-tagged M13 primer, 0.1 U/µL Taq polymerase and 1 µL of template DNA (5 ng/µL). PCR cycling was as follows: 95°C (3 min); 10

cycles of 94°C (35 s), annealing temperature (T_a) plus 10°C (-1°C/cycle) (35 s) and 72°C (45 s); 30 cycles of 94°C (35 s), 53°C (35 s) 72°C (45 s); and 72°C (20 min). A T_a of 53°C was used for M13-tagged primers (Schuelke 2000). Amplicons were co-loaded and diluted 1:30 in water, and 1 µL was added to 8.7 µL of Hi-Dye formamide (ABI) & 0.3 µL of GeneScan 500-LIZ size standard (ABI) and analyzed using an ABI 3730 sequencer. GeneMapper v.4.0 (ABI) was used to visualize and score genotypes. Genetic diversity statistics were obtained using the Excel Microsatellite Toolkit (Park 2001) (Appendix 3-1). Tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using GenePop v.1.2 web server (Raymond and Rousset 1995; Rousset 2008).

Of the 87 loci tested, 59 produced detectable peaks in at least six individuals. Monomorphism was observed in 23 loci, and seven loci had inconsistent peak patterns that could not be reliably genotyped. Of the remaining 29 polymorphic loci, five had low allele frequencies and could not be tested for HWE, and one locus deviated from HWE (Appendix 3-1). Evidence for null alleles was found for six loci using MicroChecker v.2.2.3 (van Oosterhout *et al*. 2004) (Appendix 3-1). Significant linkage disequilibrium was not detected. The number of alleles per locus ranged from 2-8 with an average of 3.52. Observed and expected heterozygosity ranged from 0-0.91 (averaging 0.336) and 0.09-0.9 (averaging 0.438), respectively.

Since M13-tagged forward primers are known to complicate PCR amplification (de Arruda *et al.* 2010) we also obtained 5' fluorescently labeled primers for 20 loci (including all that seemed potentially useful in the test panel) and assayed these in 31 individuals from northern Minnesota and Ontario. Optimization of T_a and $MgCl_2$ concentrations was performed for each locus to increase amplification success (Table 3-1). Of these 20 loci, two had low allele frequencies and four had genotype frequencies that deviated from HWE (Table 3-1). The

remaining 14 loci satisfied HWE (Bonferroni corrected $P < 0.0025$). Significant LD was detected between two loci, Fum145 and Fum160 ($P = 0.0006$). Observed and expected heterozygosity ranged from 0-0.806 (averaging 0.233) and 0-0.862 (averaging 0.346), respectively. Eleven of these loci have since been shown to be informative for analysis of population structure across seven spruce budworm populations in the Border Lakes Landscape of northern Minnesota and Ontario (Table 3-1; James *et al.* in review). In comparison with the eight SSR loci previously developed by Lumley *et al*. (2009), our reliance on an EST resource has nearly tripled the number of useful SSRs but required only a fraction of the time needed using traditional cloning techniques.

Table 3-1: Summary data for 20 EST-SSRs tested on 31 individuals of *Choristoneura fumiferana* from the Border Lakes landscape of northern Minnesota.

Locus	Label	Repeat Motif	Ta (ºC)	$MgCl_2$ (mM)	n	N_a	Range	H_o (H_e)	HWE
Fum107	VIC	$(TAA)_4$	57	2.5	31	2	133-136	0.1612 (0.2522)	0.0929
Fum120	NED	$(CTG)_6$	55	1.5	31	4	*134-148*	0.5161 (0.5684)	0.7483
Fum122	6FAM	$(TCA)_4$	59	2.5	31	3	108-114	0.1935 (0.1803)	1.0000
Fum128	NED	$(TGTT)_4$	57	2.5	31	2	124-140	0.0322	-
Fum129	NED	$(ATAA)_4$	59	2.5	30	10	*139-160*	0.2000 (0.7316)	*0**
Fum130	PET	$(TACC)_4$	55	1.5	31	2	*135-145*	0 (0.0634)	0.0168*
Fum133[a]	NED	$(GAG)_4$	53	3.5	31	2	*101-103*	0.0967 (0.0936)	1.0000
Fum137	PET	$(GCA)_6$	59	2.5	31	2	106-109	0 (0.0634)	0.0166*
Fum145	PET	$(ACT)_6$	59	2.5	31	5	*140-153*	0.2580 (0.4077)	0.0134*
Fum147	VIC	$(AAT)_4$	53	3.5	31	4	*102-109*	0.8064 (0.6409)	0.0992
Fum149	6FAM	$(TA)_7$	55	1.5	29	4	138-144	0.0689 (0.1336)	0.0359*
Fum157	VIC	$(GA)_7$	55	1.5	31	3	*147-150*	0.1612 (0.2363)	0.0097
Fum160	VIC	$(CGT)_5$	57	3.5	31	3	133-139	0.3548 (0.3484)	1.0000
Fum164	PET	$(ATA)_5$	57	3.5	31	5	*120-127*	0.1612 (0.5949)	*0**
Fum169	NED	$(AAT)_6$	55	3.5	31	4	144-150	0.0645 (0.1253)	0.0332*
Fum170	6FAM	$(ATA)_5$	59	2.5	31	3	*143-150*	0.2903 (0.4965)	0.0086*
Fum171	PET	$(GAG)_4$	59	2.5	31	1	112	0	-
Fum177	NED	$(AG)_7$	59	3.5	24	10	133-155	0.3333 (0.8617)	*0**
Fum185	6FAM	$(TCATA)_4$	59	3.5	31	5	130-140	0.5161 (0.6430)	*0.0004*
Fum187	6FAM	$(AGG)_5$	59	2.5	31	3	101-110	0.4516 (0.4394)	1.0000

Italicized locus names indicate loci that had inconsistent or low amplification success using a validation panel of 11 individuals, underlined locus names identify the 11 loci deemed most informative about population structure across seven spruce budworm populations in northen Minnesota and Ontario (James *et al.* in review), *Label* is the fluorophore used (primer sequences are identified in Appendix 3-1), T_a and $MgCl_2$ are the optimal annealing temperature and magnesium chloride concentrations, *n* is the number of individuals assayed, N_a and *Range* are the number of alleles and size ranges (ranges are italicized if allele sizes are not multiples of the repeat length suggesting the presence of indels), $H_o(H_e)$ is the observed and expected heterozygosity (only a single value if no difference), and *HWE* is the p-value for a test of deviation from Hardy-Weinberg equilibrium (italics indicate significant deviation after Bonferroni correction, $P < 0.0025$).* Loci with excess homozygosity. [a] Peaks 105 bases in length were also present in all individuals at this locus as would be expected based on information from Appendix 3-1, indicating that the size range reported here may be due to unspecific amplification.

Literature Cited

Anderson, D.P., and B.R. Sturtevant. 2011. Pattern analysis of eastern spruce budworm *Choristoneura fumiferana* dispersal. Ecography, 34: 488-497.

de Arruda, M.P., Goncalves, E.C., Schneider, M.P.C., da Costa da Silva, A.L., and E. Morielle-Versute. 2010. An alternative genotyping method using dye labeled universal primer to reduce unspecific amplifications. Molecular Biology Reports, 37: 2031-2036.

Beldade, P., van't Hoff, A., Jeronimo, M. A., and A.D. Long. 2009. Microsatellite markers associated with genes expressed in developing wings of *Bicyclus anynana* butterflies. Molecular Ecology Resources, 9: 1487-1492.

Ewing, B., Hillier, L., Wendl, M., and P. Green. 2008. Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Research, 8: 175-185.

van't Hof, A. E., Brakefield, P. M., Saccheri, I. J., and B.J. Zwaan. 2007. Evolutionary dynamics of multilocus microsatellite arrangements in the genome of the butterfly *Bicyclus anynana*, with implications for other Lepidoptera. Heredity, 98: 320-328.

Kim, K. S., Ratcliffe, S. T., French, B. W., Liu, L., and T.W. Sappington. 2008. Utility of EST-derived SSRs as population genetic markers in a beetle. Journal of Heredity, 99: 112-124.

Lumley, L. M., Davis, C. S., and F.A.H. Sperling. 2009. Isolation and characterization of eight microsatellite loci in the spruce budworm species *Choristoneura fumiferana* and *Choristoneura occidentalis*, and cross-species amplification in related tortricid moths. Conservation Genetics Resources, 1: 501-504.

Meglecz, E. 2007. MicroFamily: A computer program for detecting flanking region similarities among different microsatellite loci. Molecular Ecology Notes, 7: 18-20.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535-538.

Park, S. D. E. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. Ph.D. thesis, University of Dublin.

Pertea, G., Huang, X., Liang, F., Antonescu, V., Sultana, R., Karamycheva, S., Lee, Y., White, J., Cheung, F., Parvizi, B., Tsai, J., and J. Quackenbush. 2002. TIGR Gene Indices Clustering Tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics, 19: 651-652.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86: 248-249.

Rozen, S., and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S. (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp. 365-386.

Rousset, F. 2008. GENEPOP'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8: 103-106.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology, 18: 233-234.

Sinama, M., Dubut, V., Costedoat, C., Gilles, A., Junker, M., Malausa, T., Martin, J.-F., Neve, G., Pech, N., Schmitt, T., Zimmermann, M., and E. Meglecz. 2011. Challenges of microsatellite development in Lepidoptera: *Euphydryas aurinia* (Nymphalidae) as a case study. European Journal of Entomology, 108: 261-266.

Volney, W. J. A., and R.A. Fleming. 2000. climate change and impacts of boreal forest insects. Agriculture, Ecosystems and Environment, 82: 283-294.

Volney, W. J. A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characteristics. Global Change Biology, 13: 1630-1643.

White, J. R., Roberts, M., Yorke, J. A., and M. Pop. 2008. Figaro: a novel statistical method for vector sequence removal. Bioinformatics, 15: 462-467.

Zhang, D. X. 2004. Lepidopteran microsatellite DNA: redundant but promising. Trends in Ecology and Evolution, 19: 507-509.

Appendix 3-1: EST-based SSR information

SSR	Type	N	N_a	Range	HWE	Uniqueness	Rl	Rn	Motif	Size	Forward (5'-3')	Reverse (5'-3')	Var	Poly	Alt
Fum101	poly	9	3	*136-138*	0.0134*	Unique	3	4	aag	122	CGTTGACGAATAAAATTGTTGAA	TCAGCTGTATTACTTTGTGGTGTT	no	no	n/a
Fum102	mono	-	-	-	-	complement	3	4	tct	137	CGTAAATGCCGGTATGTGACTA	GCAACCAGTTTCGACAATACAA	no	no	n/a
Fum103	poly	9	2	163-166	-	Unique	3	5	aag	149	CTGCTAAAAGCAAGGAAGGTGT	GCAGTATGCGAACCATTCATAA	no	no	n/a
Fum104	mono	-	-	-	-	complement	3	4	gtg	120	GTACTAACGCTTAATGCCAGCC	CCGTGTACTTCAAGAAGGAGGT	yes	no	n/a
Fum105	poly	6	2	*160-161*	0.0892	unique	3,3	5,5	$(ggc)_5$TTCAGCGGT GGATTCAGCGGTG GCCTGA$(gcg)_5$	145	CTACAACTACAACCGCCCGT	GAGCTGTTGGTCGACGTTCT	no	no	n/a
Fum106	poly	8	5	*150-160*	0.0133*	unique	3	4	ata	136	AGAAAAACAAACGGCAGAACAT	TTAACATTTCATTAGCATGGCA	no	no	n/a
Fum107	poly	11	8	146-164	0.0605	unique	3	4	taa	136	CAACACAACGACCACAAACTGT	TTGCTGTGTGAATGTATGTTGC	no	no	n/a
Fum108	incon	-	-	-	-	unique	3	4	cca	102	CATAAGCATGTAACCATCCACG	CTCTTTCACCACTTCCTTCACC	no	no	n/a
Fum109	mono	-	-	-	-	unique	3	4	ggc	127	TTGTTGGTAGTTGTTGCTTTGG	TTCCATTACTGTCCGAGTCAAA	no	no	n/a
Fum110	mono	-	-	-	-	unique	4	4	tgga	149	TGCTTTGGAAATTTAACCCGTA	TCAAAAGCAGCTATCGAATACA	no	no	n/a
Fum111	poly	11	2	164-172	-	unique	4	4	ccat	148	AACGCGTCACTGCGTATAGTTA	CGCGGTCAACTCTTTTCTTTAC	no	no	n/a
Fum112	fail	-	-	-	-	unique	2,3	6,4	$(ta)_6$TTTTGTAA$(att)_4$	147	ATGTTGCTACGTCCCGTGTACT	CAGCTGAATCACACCACTAGTCA	no	no	n/a
Fum113	fail	-	-	-	-	unique	2	6	gc	136	GGCTTTTCAGAACTTCCTGATG	GCTGCTAGAGATCGACTGCATA	no	n/a	n/a
Fum114	fail	-	-	-	-	unique	3	5	ggc	139	AATGGTATCGAAATGTTAGGCG	CTCTAAAGCTACCGCCTCCTC	yes	no	n/a
Fum115	poly	11	3	135-150	0.0083*	unique	5	5	aatct	132	CCATCCTTTTGTGAATTTCTTG	CTTGATGAAGATGCCGATGTAA	no	no	n/a
Fum116	fail	-	-	-	-	unique	3	5	tat	115	CCAGAAGTATGGCCTGCTGTAT	ACCATAGCAAATTGAAGACAACT	no	no	n/a
Fum117	fail	-	-	-	-	unique	3	4	tat	129	GTGCATGCATGTCAAGAGATTT	TCATTAGAATTATGGCCAAGGAA	no	no	n/a
Fum118	mono	-	-	-	-	complement	3	4	gtt	107	GATCCACTTCGATTCGTTTACC	TTGGAACGAGAGGAAACTTTTT	no	no	n/a
Fum119	fail	-	-	-	-	unique	3	4	ctg	150	ACCGATTTCTGCTATTCTGCTG	ATAACCACTTTGCTGCTGTGC	no	no	n/a
Fum120	poly	11	4	160-169	1	complement	3	6	ctg	146	TTCTACACAGTGGGTTGTGGTC	TCCAGATACTCAGTGTCCTGCT	no	no	n/a
Fum121	mono	-	-	-	-	unique	3	4	aat	137	GGAGCTAGGATGTGGAAAACAC	AATCCCAAGCAGAAATTCACAC	no	no	n/a
Fum122	poly	11	2	126-129	1	complement	3	4	tca	109	ATGTGTTTCGTCATCGTCATTT	GCAGTACACCGAACAACAAAAA	no	no	n/a
Fum123	fail	-	-	-	-	unique	4	4	tttc	150	TTTGTAAAAATGTCACGAATAAATG	AAGGACGGACGGAAATACTAAT	yes	no	n/a
Fum124	incon	-	-	-	-	unique	3	4	gac	121	TGCTTATTCATACCCCGATGAT	CATGTCATCGCTGTCTTCATCT	yes	no	n/a
Fum125	fail	-	-	-	-	unique	3	6	tat	148	TGCTTTAAGTGTGGATGACAATG	TGTTGAATAAATTGCAAGGACA	yes	no	n/a
Fum126	fail	-	-	-	-	complement	3	4	atc	109	TTAGTCACTGTTTGCCGTCTGT	CTCACTGGGGGAGTTCTATGTC	no	no	n/a
Fum127	mono	-	-	-	-	unique	3	4	gct	109	AATGCATCGGTGCTCTATATCC	GTTGAGGGACACCTTCTGTGAT	no	no	n/a
Fum128	poly	11	4	*136-162*	0.206	unique	4	4	tgtt	125	ACAACAATATCCCACTTCCCAC	CTCAAACTGCCTGTATACCGAA	no	no	n/a
Fum129	poly	8	8	162-178	0.0448	unique	4	4	ataa	146	TATTGTCCCATTCTCCCATTTC	TGGTGTGAATTGTCCCTTGATA	no	no	n/a
Fum130	poly	11	4	*148-170*	0.0204*	unique	4	4	tacc	136	TCGTGACGCTGTGCCTACTAT	CGTAATCCGGTCCACATTTATT	no	no	n/a
Fum131	mono	-	-	-	-	unique	3	4	aag	136	TGAAGTGTCAGAAGAAGGACGA	TTTTCCAGCAATCTACGCAATA	no	no	n/a
Fum132	mono	-	-	-	-	redundant	3	5	tca	103	ATGGTCATCGTGTTGTTAGTCG	TCTAAGGGAGGAATTTGGTTCA	yes	no	n/a
Fum133	poly	8	3	123-132	1	complement	3	4	gag	107	CATGACTTTTCAATGTGGAGGA	GTAAGTTGAAGTCGTTGAACCG	no	no	n/a
Fum134	fail	-	-	-	-	unique	3	4	ata	135	CTTCAATTTTCAAACGAATTGTC	TGCTAAGTTTGGCTGAGTTGAA	yes	no	n/a
Fum135	mono	-	-	-	-	complement	3	4	taa	148	CATTCTTCGCAACATATCCTCA	TCTATCGGATACGAAATAGATACCA	no	no	n/a
Fum136	poly	10	2	142-145	0.0098*	complement	3	4	att	127	TCTTTGGGTTTTGCTATTTGGT	TTCAATGTTACGTGCTTGTCAAT	yes	no	n/a
Fum137	poly	11	2	118-121	-	redundant	3	6	gca	108	GGTTCTTCCTCCTCAACCTTCT	CAAGGATCCGTCTAAGTTCGTC	yes	no	n/a
Fum138	mono	-	-	-	-	unique	4	4	aata	114	AAACTGATCTTCATTGGTTGTTGA	TGACGTTAGGTGTCGCTGTTAT	no	no	n/a
Fum139	mono	-	-	-	-	complement	3	4	gcg	142	TAGCCGTACGTAACACATCACC	GATACTTGCGTTGTGATTTCCA	yes	no	n/a
Fum140	fail	-	-	-	-	unique	3	4	gtt	150	TATCTACTGCTGGGGTTACGCT	ATTTAACAGATTCCGCAGCAAC	yes	no	n/a
Fum141	mono	-	-	-	-	complement	3	4	aga	116	CTGGAAACTGAGGAAAGATTGG	TGGAAGTAGTTGTGGTTGTTGG	no	no	n/a
Fum142	fail	-	-	-	-	complement	3	4	cct	149	TATTAAAGTTCGGTTCACTGCG	CAAGCTCATCACCATGCTCAC	yes	no	n/a

SSR is microsatellite locus; Types are fail (failed amplification), mono (monomorphic), poly (polymorphic), or incon (inconclusive) in test panel of 11 C. fumiferana individuals; N is number of samples genotyped; Na is number of alleles; Range is size range plus 18nt M13 sequence, italics indicate loci with alleles that are not multiples of the repeat length; HWE is the pvalue for deviation from Hardy-Weinberg equilibrium, italics indicate significance after correction for multiple tests; Unique = unique (locus is unique among unigenes), complement (locus has reverse complement among unigenes suggesting paralogous gene regions, see text), redundant (locus has high similarity to unigenes that are not reverse complements also indicative of paralogous gene regions); Rlen is repeat length; Rnum is number of repeats; Motif is repeat sequence; Size is the expected PCR product length; Forward is forward primer sequence minus 18nt M13 tag, underlined if primer has variable sequence; Reverse is reverse primer sequence, underlined if primer has variable sequence; Variants indicates if the microsatellite insert contains indels and/or snps in the unigene ESTs; Poly indicates locus is polymorphic within ESTs; Alt identifies the alternative allele found within ESTs.

Appendix 3-1: (continued)

SSR	Type	N	N_a	Range	HWE	Unique	Rl	Rn	Motif	Size	Forward (5'-3')	Reverse (5'-3')	Var	Poly	Alt
Fum143	fail	-	-	-	-	complement	3	4	aag	176	GGCGGATAAGGACAAGAAAGTA	GGGTGAACATGGAGAAGACATT	yes	no	n/a
Fum144	fail	-	-	-	-	complement	2	6	cg	138	GAACAACGTGTTCATCATCGG	ATCCTAAGATCCTAGCTGGCCT	no	no	n/a
Fum145	poly	11	3	157-163	0.0389	unique	3	6	act	146	TGATCGTTTGGCATAACTCTTTT	TTGCATTTTTGTTAGTTTTCTCG	no	no	n/a
Fum146	fail	-	-	-	-	unique	3	4	aca	150	CATTCTGCGTCTCCAATCAC	TATCAGATCGTTGACCTTGGTG	no	no	n/a
Fum147	poly	7	5	120-129	0.0312	unique	3	4	aat	110	GCTGGTTTTCAGTATGACCCTT	ATTGAAGTTAAGCGTGCCAAAT	no	no	n/a
Fum148	fail	-	-	-	-	redundant	3	5	ggc	144	ACTCCTCCTTCTTGGTCTCCTT	AGAGCCTCGAGGAGCTGATT	yes	no	n/a
Fum149	poly	11	5	151-161	*0.0012*	complement	2	7	ta	141	AGCCGTTAATTGTATGATTGTAGA	GGTGGGTTTATGGCAAGTAATG	no	no	n/a
Fum150	mono	-	-	-	-	complement	3	4	aag	117	GTACAGGTGACCATCAAGAGGC	TAGCCTAATCTCATCACGCTCC	no	no	n/a
Fum151	mono	-	-	-	-	unique	3	4	aag	147	TGAGAACCATGTCATCCAGTTC	ATGAGAGCGGTGATCTTCAACT	no	no	n/a
Fum152	fail	-	-	-	-	complement	3	4	tca	139	CAAACAGGTCGACATCATCGT	TACAACCAGATCGCGTCGTACT	yes	no	n/a
Fum153	fail	-	-	-	-	unique	3	4	cat	141	GACCGCCGCTCTATTATTTATG	CCGCGTTGACTTTAATTTCATT	no	no	n/a
Fum154	fail	-	-	-	-	complement	3	4	aag	100	GAAGAAGAAGGAGGGATCAGGT	CTTGTCGAACAGAACTTGGTTG	yes	no	n/a
Fum155	poly	6	2	*140-142*	-	complement	3	4	tat	124	GAACAATGAACTTTCTTTCCACA	AAATGGTTCATTCAAATCATTCC	yes	no	n/a
Fum156	fail	-	-	-	-	unique	3	4	ggt	145	TCAACAATATAGCAACAACCGC	AGTGTACTTAGCACGAAACCCG	no	no	n/a
Fum157	fail	-	-	-	-	complement	2	7	ga	148	TTCATCGGTGCCTAGAATGATA	GAAGACGCGTGATTTTGTTAAA	no	no	n/a
Fum158	mono	-	-	-	-	redundant	3	6	atc	100	CCATTAAGTTGTAGGAGAATGACG	CAAGCCCTCTTGTTCTGAGAG	no	no	n/a
Fum159	fail	-	-	-	-	complement	3	4	tta	141	GCAAATACTTCGTTTTGTTGTTTG	CGCACATCCCCAAGTAATTCTA	yes	no	n/a
Fum160	incon	-	-	-	-	unique	3	5	cgt	138	GCGTGCTTCTCTCATTATATCG	GTCGTAACAAGGTTTCCGTAGG	no	yes	$(cgt)_4$
Fum161	incon	-	-	-	-	unique	3	5	ata	140	CACCATCCAAAGAACTGACAAA	TGGCATGGATTTATTGACACTTA	no	no	n/a
Fum162	mono	-	-	-	-	unique	3	4	ctt	147	TATGGCTTTATTCACCAGCAAC	CCTTATGAGGTGATCAGGAAGG	yes	no	n/a
Fum163	poly	11	2	127-135	1	unique	4	4	taat	118	TGTGTTGTTAATTGTTGCCATT	GGAACGTCAATGTTACGTCAAG	no	no	n/a
Fum164	incon	-	-	-	-	unique	3	5	ata	126	CAAACCGTCGAGAAATAGAAGC	TGAATGAATGAGTGATCAAGGT	yes	yes	$(ata)_1$
Fum165	poly	7	2	122-134	0.0894	unique	4	5	attt	116	AACGGCCATTCCATTACTCTTA	CACCTAATTTTCCTTATGTATCCCA	yes	yes	$(attt)_1$
Fum166	poly	7	2	140-152	-	complement	3	4	att	123	AATATTGCTTACGGCGAATCTC	GCATTATCCGACCAATTTCATT	yes	no	n/a
Fum167	fail	-	-	-	-	complement	3	4	tta	148	ATTACTGGGCTAATTGAATGCC	CGTCTAGCTTTCATTTGTCTGC	yes	no	n/a
Fum168	mono	-	-	-	-	redundant	3	4	gaa	146	AAAGAACACGAGCCTAACATCG	ATTCCAGTGTCTGTCCCGTCT	n/a	n/a	n/a
Fum169	poly	10	4	*145-168*	0.1848	unique	3	6	aat	150	CAACTCAGTAGCGAGTGTGAGG	GGGATTTACGGGTCATTCATT	n/a	n/a	n/a
Fum170	poly	11	4	159-168	0.5926	unique	3	5	ata	149	ACAGTAATAGTTTGTGGCGACG	GGGGTTTAGTCTATTTGGTGTTTG	n/a	n/a	n/a
Fum171	poly	8	2	126-129	1	unique	3	4	gag	110	CAATGTGTAGGCTCAATAGTGACA	AATCATAGGATTCCCTCCAAGC	n/a	n/a	n/a
Fum172	fail	-	-	-	-	unique	3	4	taa	149	GTTTCAAATTGTTATGCCGGTT	AACTGCAATCAAATCTCTGTGTG	n/a	n/a	n/a
Fum173	incon	-	-	-	-	unique	3	5	ggc	123	TAGACTAGGTAAGCTTGCACGG	AGACTCACAGTATTCGTCGCTG	n/a	n/a	n/a
Fum174	fail	-	-	-	-	unique	3	4	agg	139	GGAATACCTCCTCACTTTCGG	TACTGTTATGGCTGGAGCTGAA	n/a	n/a	n/a
Fum175	fail	-	-	-	-	redundant	3	4	gat	108	CAGTGGGACGTATATAGGCTGG	TTTTCTCAGTGTTTGTCTTGCG	n/a	n/a	n/a
Fum176	incon	-	-	-	-	unique	4	5	tgtc	134	GGAACCCTTATAGTTTCGCCAT	GAATTGTACCATTTTGTTGGCA	n/a	n/a	n/a
Fum177	fail	-	-	-	-	redundant	2	7	ag	146	TCTAACTTGTCAAACGGACACG	GGTCGACATTACTAGAGCTCGC	n/a	n/a	n/a
Fum178	mono	-	-	-	-	unique	3	4	gtt	106	AGCTCTCTCAATATGCCCTTTG	TCAAGTACCAAGCCCACCTATT	n/a	n/a	n/a
Fum179	fail	-	-	-	-	unique	3	5	att	111	TGAACTGAGTGAATGAAAATCCA	CAACAACAACAAATAACGCTGA	n/a	n/a	n/a
Fum180	mono	-	-	-	-	unique	3	4	tta	135	AGTCGGCATTTTGCTTAATTTC	AAACAAATGGCGTTCTCTTCAT	n/a	n/a	n/a
Fum181	mono	-	-	-	-	unique	3	4	tga	140	CAAAAGATTTATCGCCCAGTCT	ATACAGCTGTTCATGTTCACGG	n/a	n/a	n/a
Fum182	poly	11	6	*141-174*	0.0467*	unique	2	6	at	149	GCACGAACGTATTAGTGCAAGA	TTGCACAATTGATACAGAACCTT	n/a	n/a	n/a
Fum183	poly	11	3	160-166	0.2805	unique	3	4	cca	148	TTGCAATGTGTGTGTAGGTATG	CTGGTCGGGCACTATCAAAT	n/a	n/a	n/a
Fum184	mono	-	-	-	-	unique	3	4	tga	123	TGGTTATCCTTCTGGTAACATGG	ACAGGCAGATATAGCCCACAGT	n/a	n/a	n/a
Fum185	poly	11	3	*152-160*	0.2812	unique	5	4	tcata	136	TTTGGGTTGTGAATAGGTGTCA	ATAAGGCAGTGGAGTGAGGGT	n/a	n/a	n/a
Fum186	mono	-	-	-	-	unique	3	4	taa	102	AAAAGGCAAACGCAAATAAAAA	AAAAGTTTTTCGAGCCGATTC	n/a	n/a	n/a
Fum187	poly	11	5	*118-129*	1	unique	3	5	agg	109	CGTGACAGATAAGAACATCCGA	TCATCATCAGTCAGTATCAGCG	n/a	n/a	n/a

SSR is microsatellite locus; Types are fail (failed amplification), mono (monomorphic), poly (polymorphic), or incon (inconclusive) in test panel of 11 C. fumiferana individuals; N is number of samples genotyped; Na is number of alleles; Range is size range plus 18nt M13 sequence, italics indicate loci with alleles that are not multiples of the repeat length; HWE is the pvalue for deviation from Hardy-Weinberg equilibrium, italics indicate significance after correction for multiple tests; Unique = unique (locus is unique among unigenes), complement (locus has reverse complement among unigenes suggestive of paralogous gene regions, see text), redundant (locus has high similarity to unigenes that are not reverse complements, also indicative of paralogous gene regions); Rlen is repeat length; Rnum is number of repeats; Motif is repeat sequence; Size is the expected PCR product length; Forward is forward primer sequence minus 18nt M13 tag, underlined if primer has variable sequence; Reverse is reverse primer sequence, underlined if primer has variable sequence; Var indicates if the microsatellite insert contains indels and/or snps in the unigene ESTs; Poly indicates locus is polymorphic within ESTs; Alt identifies the alternative allele found within ESTs.

Chapter 4

Two's company, three's a crowd: population structure and asymmetry in a three-way hybrid zone between spruce budworm species in the Canadian Rocky Mountains

Introduction

Hybrid zones provide a multifaceted window into the nature of species boundaries (Hewitt 1988). These areas of genetic interaction between species or distinct lineages reflect a balance between selection against hybrids, gene flow between species, and ecological factors that in different contexts result in very different evolutionary trajectories of the species involved (Barton and Hewitt 1985; Hewitt 1988; Abbott *et al*. 2013). For instance, "hybrid swarms" occur when selection against hybrids is weak or nonexistent, and are typically associated with novel or intermediate habitats compared to those of the parental species (Anderson 1948; Seehausen 2004; Nolte and Tautz 2010). Further stabilization through reproductive isolation from parental species can then establish hybrid lineages as evolutionarily independent entities (Buerkle *et al*. 2000; Seehausen 2004; Mallet 2007; Nolte and Tautz 2010). At the other extreme, the counteracting forces of dispersal and selection against hybrids, whether ecologically dependent or not, can result in a bimodal distribution of genotypes where hybrids are rare or frequently assimilated into parental populations (Jiggins and Mallet 2000; Smith *et al*. 2013). In these environmental clines or "tension zones" (Barton and Hewitt 1985; Hewitt 1988), assortative mating among parental populations due to reduced hybrid fitness can then reinforce species boundaries and drive species divergence (Butlin 1987; Servedio and Noor 2003).

However, hybrid zones are rarely comprised of exclusively distinct hybrid classes, more often showing considerable variability and intermediacy with semi-permeable barriers to gene flow that relate to the amount of divergence that has occurred between species (Abbott *et al*.

2013). Several factors can obscure the relatively simple task of identifying hybrids in nature. Patterns similar to introgression can result from retained ancestral polymorphism that may be prevalent early in speciation (Lexer *et al*. 2006; Stolting *et al*. 2012). This is because the time for genetic drift and selection to sort alleles into particular lineages (otherwise known as lineage sorting) is reduced in taxa that have undergone a recent divergence. Admixed individuals are also commonly indistinguishable from parental populations owing to the porous nature of species boundaries (Wu 2001; Minder and Widmer 2008; Stolting *et al*. 2012). Although the development of molecular techniques over the last three decades has mitigated many of these challenges, identifying hybrids, particularly advanced-generation hybrids, continues to be difficult when genetic differentiation between species is weak and a limited number of loci are available (Anderson and Thompson 2002; Buerkle 2005; Fitzpatrick 2012). Recent advances in high-throughput sequencing of reduced-representation genomic libraries, such as Genotyping-by-Sequencing (GBS), now provide an opportunity for the rapid discovery and genotyping of genetic markers sampled randomly across the genome (Davey and Blaxter 2010; Davey *et al*. 2011), and mark a new era in the study of hybrid zones of non-model organsims (Hohenlohe *et al.* 2011; Stolting *et al*. 2012).

The spruce budworm, *Choristoneura fumiferana* (Clemens), species complex is one of North America's most economically important groups of forest pests, containing from 8 to 15 species or biotypes (Harvey 1985a; Volney and Fleming 2007; Lumley and Sperling 2011a). Species in the complex are divided geographically by strong affinities to either eastern boreal or western montane ecosystems (Stehr 1967; Harvey 1985a), with vast areas of sympatry between species differing in sex pheromone composition (Sanders *et al*. 1977) and larval host associations (Stehr 1967; Harvey 1985a). Although extensive work has identified weak reproductive barriers

between putative species within controlled environments (Smith 1953, 1954; Campbell 1958, 1962, 1967; Stehr 1967; Sanders 1971a, 1971b; Sanders *et al*. 1977; Liebhold *et al*. 1984; Volney *et al.* 1984; Volney and Liebhold 1985; Harvey 1961, 1997; Nealis 2005), the incidence of naturally occurring hybridization within the group is not well understood. Given the potential for the spread of advantageous traits between species (Morjan and Rieseberg 2004; Parsons *et al.* 2006; Martin *et al*. 2006) and the adaptive divergence of established hybrid populations (Seehausen 2004; Rieseberg *et al.* 1999, Nolte and Tautz 2010), knowledge of naturally occurring hybridization among spruce budworm species is of the utmost importance to the management of forest resources.

The only comprehensive examination of a natural hybrid zone within the complex is that between *Choristoneura occidentalis* Freeman and *Choristoneura retiniana* (Walsingham) in Oregon and northern California. The former was recently renamed *Choristoneura freemani* Razowski (Razowski 2008), but we retain the use of *C. occidentalis* to maintain congruence with the vast literature that has been accrued on this species since its description in 1967 (Freeman 1967). Although hybrid larvae could be identified in this hybrid zone by their morphological intermediacy relative to parental populations (Liebhold *et al*. 1984; Volney *et al*. 1984), identification of other hybrids within the species complex is usually difficult as morphological characters do not reliably distinguish species (Harvey 1985a). In eastern North America, Deverno *et al*. (1998) provided the first molecular evidence of hybridization between wild populations of *C. fumiferana* and *Choristoneura pinus* Freeman. Earlier work proposed that the broadly sympatric populations of these two species were reproductively isolated by differences in habitat preference, phenology, and cross-attraction (Smith 1953). However, by genotyping 17 randomly amplified polymorphic DNA (RAPD) genetic markers, Deverno *et al.* (1998) were

able to identify hybrid specimens from multiple populations, providing further support for incomplete barriers to gene flow and highlighting the utility of molecular approaches in the study of naturally occurring hybrids between these cryptic species. In addition, phylogenetic discordance in nuclear and mitochondrial ancestry in areas where the ranges of eastern boreal and western montane species overlap suggests that hybridization between these largely parapatric groups may also occur in nature (Sperling and Hickey 1994; Lumley and Sperling 2011a, 2011b; Brunet *et al*. 2013).

In this study, we use single nucleotide polymorphisms (SNPs) assayed by GBS to investigate a putative hybrid zone between three of the most economically important species in the spruce budworm species complex, *C. fumiferana*, *C. occidentalis*, and *Choristoneura biennis* Freeman. *Choristoneura fumiferana* is a boreal species that ranges from the maritime provinces of Canada west to the boreal cordilleran forests of east-central Alaska and southern Yukon (Shepherd *et al*. 1995). *Choristoneura biennis* has a two-year life cycle, contrasting with the one-year cycle of the other two species, and occupies a subalpine niche that extends from eastern British Columbia (BC) to the Rocky Mountains, forming a wedge between *C. fumiferana* to the north and *C. occidentalis* in the south (Shepherd *et al*. 1995). *Choristoneura occidentalis* is distributed at low elevations over much of southern BC and the Pacific Northwest United States and extends into southern Arizona and New Mexico (Harvey 1985a; Shepherd *et al.* 1995). These three species contact each other along the eastern foothills of the Rocky Mountains where the ranges of their primary hosts *Picea glauca*, *Picea engelmannii* and *Pseudotsuga menziesii* overlap (Stehr 1967; Shepherd *et al*. 1995).

We conduct a population genetic analysis of the region of potential interaction between the three *Choristoneura* species to: 1) characterize the geographical location and extent of

contact between these species, 2) identify hybrids, and 3) determine the rate and direction of gene flow between eastern and western species across the area. Existing evidence suggests that hybridization is not hindered by post-zygotic reproductive barriers between these species (Campbell 1958, 1962, 1967; Sanders 1977; Harvey 1997), and that gene flow is most likely to follow a west-to-east direction due to asymmetry in cross-attraction between eastern and western spruce budworms, differences in the weight of eggs oviposited by their reciprocal F1 hybrids, and prevailing wind direction (Sanders *et al.* 1977; Harvey 1983b; Volney 1985). Accordingly, we expect hybrids between eastern and western species to reflect primarily eastern genomic backgrounds with introgressed genetic material from western populations as gene flow in this direction has commonly been shown to occur between resident and invading populations of other species (Sperling and Spence 1991; Currat *et al*. 2008; Petit and Excoffier 2009). The geographical proximity of *C. fumiferana* to *C. biennis,* and of *C. biennis* to *C. occidentalis,* is also expected to predispose these species pairs to a higher frequency of hybridization than between *C. fumiferana* and *C. occidentalis*. An assessment of the extent and nature of admixture and gene flow between these species will strengthen our understanding of the processes that contribute to species boundaries and provide a basis for future work on ecological and genetic factors that contribute to reproductive isolation between species.

Methods

Sample collection

We used 486 adult specimens (moths) from 45 locations (Table 4-1) throughout western Canada. Of the 45 locations six were included to represent reference populations for each of the three species *C. fumiferana* (Bs and Ed), *C. biennis* (Mr and Mb), and *C. occidentalis* (BR and

P2). These six reference populations were geographically situated at the periphery of the area sampled and in areas where the predominant conifer species corresponded with that of each species' primary host. In addition, individuals from a single reference population for *C. occidentalis* (P2) were previously shown to have *C. occidentalis* ancestry using both mitochondrial sequence and eight nuclear microsatellites (Chapter 2). A combination of by-hand (hand-net or hand-picked and reared) and trapping (pheromone and light traps) methods were employed for collections. Pheromone traps were green unitraps (Contech, Delta, BC) baited with a *C. fumiferana* sex pheromone (95:5 E, Z-11-tetradecenal) flex-lure (Contech, Delta, BC) and a strip of Vaportape II (Hercon Environmental, Emigsville, PA). Specimens were frozen at -70°C upon return to the lab and held at this temperature until further use.

DNA extraction and 'Genotyping-by-Sequencing'.

DNA was extracted from the thorax and legs of 486 moths using a DNeasy Blood & Tissue DNA purification kit (Qiagen) with bovine pancreatic ribonuclease A (RNAseA) (Sigma) treatment. Following elution, DNA was ethanol precipitated and resuspended in millipore water. *PstI-MspI* GBS libraries (96-plex) were prepared by the Institut de biologie intégrative et des systèmes (IBIS) at the Université Laval (Quebec City, QC) using the protocol of Poland *et al*. (2012). These enzymes are not sensitive to DNA methylation and cut relatively infrequently owing to the long recognition sequence of PstI. Consequently, while fewer genomic regions will be represented within the libraries, this should allow for greater read depth that will be beneficial in genotype calling. Multiplex barcode sequences (4-8 bases) are listed in Appendix 4-1. Following the final amplification of the pooled, adapter-ligated restriction fragments, 600ng of each amplified library was normalized to remove the repetitive fraction by treatment with

duplex-specific nuclease (Zhulidov *et al.* 2004). Finally, an additional PCR step using a selective reverse primer that extended a single base (C) into the insert past the 3' restriction site was used to selectively amplify one-quarter of the total number of fragments and thereby increase the read depth of the sequenced fragments (Sonah *et al*. 2013). Single-end sequencing (100 bp reads) of these libraries was then performed in three lanes of an Illumina HiSeq2000 at the McGill University-Génome Québec Innovation Centre in Montreal, QC.

Based on knowledge of genome size (~516.4 ±5.5 Mb, Cusson *pers. comm.* 26 July, 2012) and base frequencies (GC-content = 37.6%) we expect the number of tags per sample to be ~31,600 using PstI (RAD Counter, https://www.wiki.ed.ac.uk/display/RADSequencing/Home). This estimate, once scaled for the desired read depth (50X) to allow greater precision in genotype calls, and the plexity (96-plex) of the GBS libraries, requires 152 million sequenced reads per flowcell lane to sequence all possible tags, well below the average read output of a HiSeq2000 sequencing platform (187 million reads per lane, http://www.illumina.com/systems/hiseq_comparison.ilmn).

SNP discovery and genotyping

FASTQ-formatted GBS data were analyzed using several publicly available bioinformatic tools. TASSEL-GBS v. 4.0 (Glaubitz *et al.* 2014) was used for sequence quality filtering, and for SNP discovery and genotyping. Reads were filtered for those with 5' matches to one of the barcode sequences plus the PstI cut site, and adapter dimers and reads with ambiguous base calls were removed. Reads were split into individual-specific pools according to their barcode sequence, and then trimmed of their barcode, partially-digested and common adapter (if present) sequences, and truncated to 64 bases. Sequences shorter than 64 bases were filled to 64

bases with polyA. A reference set of tags was then compiled by retaining only reads with a minimum q-score of 10 and occurring at least twice times across all individuals. Tags (minus polyA tail) were then aligned using the Burrows-Wheeler Aligner v. 0.6.2-r126 (BWA) (Li and Durbin, 2009) to 30,517 scaffolds of the spruce budworm draft genome assembly (Spruce Budworm Consortium, 19 April, 2011) to obtain positional information. A maximum of 4% sequence divergence and a one base gap per aligned tag was allowed. Prior maximum estimates of 2.7-2.9% sequence divergence among the three *Choristoneura* species using mitochondrial cytochrome oxidase subunit I (Sperling and Hickey 1994) suggest that this cut off should be sufficient to allow alignment of homologous nuclear regions in the genomes of these species without introducing significant alignment error.

Following the compilation of the reference tag set, all 64-base tags for each individual that perfectly matched a reference tag were retained for SNP discovery and genotyping. SNPs having a minimum minor allele frequency of 0.01 (default) and minimum locus coverage of 0.1 (default) across all individuals were identified between the reference genome and reference tags aligning to the same position. SNPs aligning to the same position but residing on opposite strands were merged. The average read depth and global heterozygosity estimate for each locus was then generated using VCFtools v. 0.1.12a (Danecek *et al*. 2011) for analysis using the statistical framework of Chenuil (2012). This framework determines the read depth (x^*) required per individual to attain >95% probability in homozygote genotype calls for each locus. Subsequently, all loci for which there was less than 5% missing data and i) the number of reads per sample (in greater than 5% of samples) was less than x^*; or ii) were either completely homozygous (for the same allele) or heterozygous across all samples, were removed from the dataset. Loci and individuals with greater than 5% missing data were discarded from the dataset

and a minor allele frequency of at least 0.05 was imposed to retain SNPs suitable for population analysis. Finally, filtering for SNPs with low levels of linkage disequilibrium (LD) (< 0.2 over 500000 bases within scaffolds) was conducted using the composite LD statistic in SNPrelate v.9.14 (Zheng *et al*. 2012).

Genetic diversity and differentiation

We assessed the extent of deviation from Hardy-Weinberg equilibrium separately for each reference population. Deviation from HWE was assessed using VCFtools. The resulting p-values were adjusted to account for a false-discovery rate (FDR) of 5% using QVALUE *v.*1.34.0 (Dabney and Storey). GenAlEx v 6.5 (Peakall and Smouse 2012) was used to quantify the genetic differentiation between species (with reference populations grouped) (F_{rt}), and the genetic differentiation between reference populations within species (F_{sr}) by analysis of molecular variance (AMOVA). We also calculated levels of inbreeding (F_{is}) in all populations, as well as the observed and expected heterozygosity (H_o and H_e, respectively), and used AMOVA to assess pairwise population genetic differentiation across all populations. AMOVAs were run using 999 random permutations of the data to establish significance thresholds for testing departures from random mating.

Population Structure

To assess population structure across the hybrid zone, individuals were assigned to one or more genetically distinct populations (K) using Structure v. 2.3.4 (Pritchard *et al*. 2000, Falush *et al*. 2003) assuming admixture between genetic clusters and correlated allele frequencies due to gene flow or shared ancestry (Falush *et al.* 2003). Following 10,000 generations of 'burn-in',

40,000 Markov-chain Monte Carlo (MCMC) generations were used for each value of *K* from 1-10. This was replicated 10 times for each value of *K* and the most likely number of clusters was determined using the method of Evanno *et al*. (2005), as implemented in Structure Harvester v. 0.6.93 (Earl and vonHoldt 2012) and by comparison of the mean log probability of the data at each value of K. Posterior mean estimates of cluster membership coefficients (q-scores) were then averaged over all replicates for each analysis following permutation with Clumpp v.1.1 (Jackobson and Rosenberg 2007).

We also used discriminant analysis of principal components (DAPC) in *adegenet* v. 1.3-9 (Jombart *et al.* 2008; Jombart *et al*. 2010.) to assess population structure. First, individuals were sequentially grouped into 1-45 clusters while retaining all principal components (PCs) and the optimal number of clusters was determined by comparison of the Bayesian Information Criterion (BIC) for each clustering solution. To avoid over-fitting of discriminant functions by using too many PCs, assignment to clusters was initially run with 153 PCs ($1/3^{rd}$ of the total number of PCs) and simulated 100 times using *optim.a.score* to determine the optimal number of PCs. Each individual was then probabilistically assigned to each cluster using the first three PCs.

Hybrid identification

To determine which types of hybrids predominate in areas of interaction in this putative three-way hybrid zone, we used *HIest* v. 2.0 (Fitzpatrick 2012). Loci with fixed allele frequency differences (*i.e.* $\delta = 1$) between reference populations of *C. occidentalis* and *C. fumiferana*, as well as between those of *C. biennis* and *C. fumiferana,* were selected for hybrid analysis. Because there were no fixed loci between *C. occidentalis* and *C. biennis*, the loci with allele frequency differences greater than 0.5 were used to identify hybrids between these species. The

likelihood of each of hybrid class possible within the first two generations of admixture between each pair of the three species (*eg.* parental, F1, F2, or backcross) was then deduced for each individual, and the most likely class was identified. The best class was then accepted if its likelihood was more than two log-likelihood units greater than the next best class, and if its likelihood was within 1 log-likelihood unit of that of a joint maximum-likelihood estimate (MLE) of each individual's hybrid index (*S*) (*i.e.* the proportion of the genome deriving from a single parental species) and interspecific heterozygosity (H_I) (*i.e.* the proportion of heterozygous genotypes with an allele from each parent) (Fitzpatrick 2012). *S* and H_I in the MLE were estimated using 500 MCMC iterations of *HIest's* simulated annealing algorithm with all other options set to default settings.

Patterns of gene flow

Gene flow between species was investigated using the coalescent-based program Migrate-n v. 3.5.1 (Beerli and Felsenstein, 2001). Migrate-n jointly estimates mutation-scaled effective population sizes (Θ) and past mutation-scaled migration rates (*M*) between populations using Bayesian inference (Beerli 2006), and can evaluate alternate gene flow models using Bayes factors (Beerli and Palczewski 2010). Asymmetric (1A, 1B), symmetric (2A, 2B), and panmictic (3) gene flow models were examined between species treating *C. biennis* and *C. occidentalis* as separate (1A, 2A) populations or as a single panmictic population (1B, 2B). For each analysis, 50 individuals were subsampled from each species with species denoted by genetic clustering at K=3 using Structure. Migrate was run using the SNP model with the transition/transversion ratio (1.59) and base-frequencies (A: 0.20, C: 0.30, G: 0.30, and T: 0.20) defined as constants for all loci. These were determined directly from the whole dataset. Starting parameters for Θ and *M*

were set as 0.01 and 1000, respectively. Mutation rate (μ) was held constant among loci. Population parameters were sampled using a Metropolis-Hastings MCMC algorithm and uniform prior distributions for Θ and M of 0-0.1 and 0-10000, respectively. Marginal likelihoods of the posterior probability distributions for each locus were calculated using the thermodynamic integration option with a static heating scheme consisting of four Markov chains running at different temperatures (1, 1.5, 3, and 100000). Parameter values were combined over three replicated long chains sampled at increments of 20 steps along a single long Markov chain consisting of 500,000 sampled parameter values after 2,000,000 burn-in steps. Finally, estimates of the number of migrants per generation (N_em) were calculated by multiplying the modal estimate of M by the modal estimate of $\Theta/4$.

Results

Sample collection and Genotyping-By-Sequencing

An average of 10 samples (5-17) was processed per location (Table 4-1). Because sampling was predominantly pheromone trap based, the majority of samples were males with the exception of 13 females and 35 individuals of unknown sex. Ten samples from a *C. occidentalis* population in Utah were included during SNP discovery for a more comprehensive survey of SNPs between species, but were excluded from later genetic analyses because the population is geographically disjunct from the hybrid zone. Accordingly the GBS sequencing statistics reported below are based on 496 individuals rather than 486. Among all individuals, a total of 985,152,090 reads representing 11,188,915 unique sequences passed our quality filters and had a minimum of 2-fold coverage. The proportion of unique reads that aligned unambiguously to the spruce budworm reference genome was 25.2% (2,824,516 unique reads), and the average total

number of reads mapped per individual was 1,908,894 (range 39,896-10,514,023). The incompleteness of the spruce budworm reference genome may account for the low number of aligned reads. Five individuals had very low sequencing coverage (*i.e.* number of mapped reads was less than the one percentile of all individuals), but were discarded in subsequent filtering steps.

A total of 128,241 SNPs were found through alignments of the set of unique reads of each sample against the reference genome that passed our TASSEL-GBS SNP discovery filters. From these, 13,232 SNPs were found to have both locus and individual call rates greater than 95%, and read depths surpassing thresholds required for 95% probability in homozygous genotype calls (average read depth threshold (x^*) = 1.1; range 0-14.2). Among these the average read depth across all individuals was 74.9 reads (range 7.6-246.9), and the average apparent level of heterozygosity was 0.08 (range 0-1). Further filtering for SNPs with a minor allele frequency of 0.05 that were not completely heterozygotic or homozygous across all individuals for the same allele resulted in 3245 loci. Finally, 2277 SNPs had overall low levels of pairwise LD with other SNPs found in adjacent stretches of scaffold sequence.

Genetic diversity and genetic differentiation

We detected significant deviations from HWE for just 29 loci within a single reference population (BR), all of which resulted from excesses in the heterozygous genotypes. Since this was not apparent in other reference populations, all loci were retained for further analysis. Observed (mean H_o = 0.202; range 0.173-0.269) and expected (mean H_e= 0.198; range 0.169-0.249) levels of heterozygosity across all populations were small and very similar (Table 4-2). Levels of inbreeding were also negligible across all populations and approached those expected

under HWE (mean F =-0.053; range -0.144-0.092; Table 4-2). The occurrence of negative inbreeding coefficients across most populations suggests that many loci may have resulted from the alignment of paralogous genomic regions. However, similar levels of F are observed even after accounting for paralogs by filtering out loci with greater than 50% heterozygosity. The genetic differentiation between reference populations of the three species was significant (F_{rt}=0.306, p=0.001) compared to that between reference populations within species (F_{sr}=0.005, p=0.233) suggesting that the reference populations adequately capture the overall differentiation between the three species. Patterns of pairwise genetic differentiation confirm this, with reference populations having greater differentiation from those of the other species. However, the differentiation is much greater between reference populations of eastern and western species (F_{st} = 0.37-0.40, p<0.0001) than between those of the two western species (F_{st} = 0.03-0.04, p<0.06). In general, the genetic differentiation between the *C. fumiferana* reference populations and a fewer other eastern populations was similar to that between most western populations, including that between the reference populations of the two western species (Appendix 4-2).

Population structure

Bayesian inference of population genetic structuring using Structure resulted in strong support for just two genetic clusters (K=2, eastern and western) with a ΔK value more than three orders of magnitude greater than for larger values of K, and the mean log probability of K values larger than two reaching a plateau (Figure 4-1). At this level of population structure, only four individuals exhibited evidence of significant admixture between clusters (*i.e.* q-score <90%): two individuals from WL (8918 and 9125), and single individuals from each of CL (7136) and SR (7223) (Figure 4-2; Appendix 4-1).

To further examine the potential basis for the taxonomic recognition of *C. biennis* and *C. occidentalis*, population substructure was explored within the strongly supported eastern and western genetic clusters. A hierarchical approach was used in which Structure was rerun for K = 1-6 separately for individuals from each of the two genetic clusters that had ancestry coefficients greater than 90%. This demonstrated only very weak support for population substructure in western genetic cluster, where ΔK suggested two subpopulations within the western cluster (ΔK = 1004.1) but very little difference in the mean log probabilities across all values of K (Figure 4-3). Admixed ancestry between these two western subpopulations was evident in 32 individuals distributed throughout much of BC and the Rocky Mountains (Figure 4-4). Similar clustering of individuals was obtained using DAPC (K=3, BIC = 2472.7, Figure 4-5).

Hybrid identification

The number of loci with fixed allele frequency differences between reference populations was 64 for *C. biennis* and *C. fumiferana* and 62 for *C. occidentalis* and *C. fumiferana*. Of these, 57 were in common across both comparisons. On the other hand, between *C. biennis* and *C. occidentalis* there were no loci with fixed differences and only eight loci with allele frequency differences greater than or equal to 0.5 (Appendix 4-3). The joint maximum-likelihood estimates (MLE) of interspecific heterozygosity (H_I) and hybrid index (S) resulted in most individuals having low H_I and S estimates less than 0.25 or greater than 0.75 using loci fixed between eastern and western species, which suggests a predominance of backcrossed hybrids (Figure 4-6a and b). In contrast, estimates of H_I and S using loci with significant allele frequency differences between the western species suggest that the hybridization is much more prevalent (Figure 4-6c). The simple classification model predicted 40 F2 and 169 backcrossed hybrids between *C. biennis*

and *C. fumiferana* among all individuals, 49 F2 and 188 backcrossed hybrids using fixed loci between *C. occidentalis* and *C. fumiferana* among all individuals. However there were only 3 F2 hybrids between *C. biennis* and *C. occidentalis* considering only individuals of western ancestry (Q >95%). However, none of these hybrids were statistically supported. In most cases this was because the likelihoods of the simple classification model and the MLE were too different, however a single individual in the *C. occidentalis* x *C. fumiferana* analysis and most individuals (250) in the *C. biennis* x *C. occidentalis* analysis could not be classified using the simple model.

Patterns of gene flow

Asymmetry in migration between *C. fumiferana* and the western cluster with *C. biennis* and *C. occidentalis* populations treated separately was the most likely gene flow model evaluated between these species (approximate Bezier log marginal likelihood = -12895). In the three-population full asymmetry model, gene flow between *C. fumiferana* and either western species was reduced compared to that between the two western species, particularly gene flow from *C. occidentalis* into *C. biennis*, although these differences were not significant as the 95% support limits in the number of effective migrants per generation overlapped between these two comparisons (Figure 4-7). Regardless, gene flow is greater from populations of *C. occidentalis* into populations of *C. fumiferana* and *C. biennis* than in the opposing directions (Figure 4-7). In addition, differences in the direction of gene flow between *C. fumiferana* and *C. biennis* populations were negligible, with gene flow only slightly greater from *C. biennis* populations to *C. fumiferana* populations.

Discussion

Species status of C. fumiferana, C. biennis *and* C. occidentalis

Inter-specific matings between *C. fumiferana*, *C. biennis*, and *C. occidentalis* are well documented within laboratory studies, but evidence for natural hybridization between these species has remained elusive. Here, we used a genomic approach to characterize the extent of hybridization between these species in the foothills of the Canadian Rocky Mountains. Our results suggest that there are two strongly differentiated genetic clusters in this region with highly correlated east-west geographical separation that is in agreement with previous work (Stehr 1967; Shepherd *et al*. 1995). These clusters correspond to the eastern species, *C. fumiferana,* and a combination of the two western species, *C. biennis* and *C. occidentalis* (Figure 4-2). Weak population substructure within the western genetic cluster weakly supported different ancestries for the reference populations of *C. biennis* and *C. occidentalis*, with separate clusters for each species. The *C. biennis* reference populations clustered with populations distributed primarily in BC and the Rocky Mountains at more northern latitudes, whereas those of *C. occidentalis* clustered with populations distributed primarily in southeast BC and along the eastern side of the Rockies in Alberta (Figure 4-4). Similar distributions have been identified with regard to bβ and oβ mitochondrial haplotypes in individuals of western ancestry (Lumley 2010). This suggests that the geographically disjunct population of the *C. occidentalis* cluster located at Merritt, BC may represent a northern extension of *C. occidentalis* populations occurring further south and extending into the Rocky Mountains of southern Alberta and BC. Regardless, the weak support for the substructure observed within the western cluster despite using a genome-wide approach can attest to the close evolutionary relationship of *C. biennis* and *C. occidentalis*.

Several lines of evidence support revision of the species status of *Choristoneura biennis*. Species are often defined as reproductively isolated lineages (Mayr 1969; Coyne and Orr 2004) or those that maintain genotypic separation or genomic integrity in the face of gene flow (Mallet 1995; Sperling 2003). Here, however, the continuous distribution of genotypes among these western species, low estimates of genetic differentiation that are not consistent with typical levels of differentiation between species, andweak support for substructure within the western genetic cluster, clearly do not fit either of these species concepts. Instead, it is conceivable that *C. biennis* may represent a northern extension of *C. occidentalis* with some genetic influences from *C. fumiferana* based on evidence here and in previous work for admixture between these species (Sperling and Hickey 1994; Gray *et al.* 1995; Shepherd *et al*. 1995; Lumley and Sperling 2011a, 2011b). Other evidence also challenges the taxonomic treatment of *C. biennis* and *C. occidentalis* as distinct species, including the inherent plasticity of their diapause characteristics (Harvey 1967; Nealis 2005), shared larval host preferences (Stehr 1967; Harvey 1985a; Brunet *et al*. 2013), and consistent lack of phylogenetic distinction between species (Sperling and Hickey 1994, 1995; Lumley and Sperling 2011a, 2011b; Bird 2013). Consequently, there is no longer any compelling evidence justifying *C. biennis* as a separate species.

Patterns of hybridization

Support for recent hybridization was conflicted between analyses. Analysis with Structure at K=2 clearly identified four individuals with significant admixture between eastern and western genetic clusters (Figure 4-2), as well as extensive admixture between subclusters within the western genetic cluster (Figure 4-4). Hybrids between eastern and western budworm species were found in three locations (WL, CL, and SR) east of the Rocky Mountains and north

of Hwy 1, and resembled F1 (n=2) and backcross (n=2) individuals based on their admixture proportions. The results of HIest's MLE model showed a similar pattern based on the relationship between interspecific heterozygosity and hybrid index (Figure 4-6a and b). However, HIest's simple classification of individuals into these hybrid types failed to find statistical support for these classifications. This may reflect limitations of HIest in characterizing hybrids as its tests are designed foremost to identify early generation hybrids, but are susceptible to high false-positive rates and the misidentification of hybrid types as parentals with low numbers of ancestry informative markers (Fitzpatrick 2012). In addition, interpretations based upon loci with uncertain parental allele frequencies (*eg.* δ=0.5 between *C. biennis* and *C. occidentalis*) will inevitably lead to poor discriminatory power of hybrid classes (Fitzpatrick 2012), suggesting that our choice of reference populations for distinguishing between *C. biennis* and *C. occidentalis* may not have been ideal. Although the identification of hybrid types between these species might have been better facilitated using populations sampled in proximity to the core of each species' range (*eg. C. occidentalis* = Pacific Northwest United States; *C. biennis* = northwestern BC), introgression from other sympatric *Choristoneura* (*e.g. Choristoneura retiniana* (Walsingham) and *Choristoneura orae* Freeman, respectively) in those areas would likely lead to further difficulties.

Processes contributing to divergence and speciation

The contrasting patterns of hybridization between these three species reveal a reproductive asymmetry in their interactions. The low frequency of recent hybrids across the region of potential contact suggests strong reproductive barriers between *C. fumiferana* and both western species, and an absence of barriers between the two western species. Additionally,

results from Migrate indicate an asymmetry in long-term gene flow across the hybrid zone and suggest differences in barriers to gene flow between these species.

Pre-mating barriers may confer some isolation between these species since subtle differences in cross-attraction are known (Sanders 1971a, Sanders *et al*. 1977). Prior hypotheses have suggested that differences in cross-attraction between *C. fumiferana* and *C. occidentalis* in the wild could result in asymmetric gene flow in an eastward direction since *C. fumiferana* females are more receptive to mating with *C. occidentalis* males than *C. fumiferana* males with *C. occidentalis* females (Sanders 1977; Volney 1985). However, although estimates of N_em between *C. fumiferana* and *C. occidentalis* were greater from populations of the latter into those of the former, all estimates between eastern and western species had overlapping 95% confidence intervals and suggest that significant differences in the directionality of gene flow were not evident. In addition, observations of such differences in cross-attraction where eastern and western spruce budworm species are sympatric in nature have yet to be demonstrated.

Differences in egg weight between species, as well as phenological synchrony with hosts have also been implicated in population growth and survival within spruce budworm biotypes (Volney and Fleming 2007). Disruption of either of these traits due to hybridization could lead to lowered fitness in admixed individuals. However, this is not apparent in other hybrid zones between spruce budworm species. In fact, in a hybrid zone between *C. occidentalis* and *Choristoneura retiniana*, hybrids were able to rapidly adapt to new habitat conditions, such as mixed stands or different phenological schedules (Volney *et al.* 1983; Volney and Liebhold 1985) suggesting that hybridization may confer greater survivability. Also, Harvey (1997) observed larger egg weights in some hybrid combinations indicating that such interactions in nature could confer greater survival of hybrids than parental populations in harsh environments.

This is because spruce budworm larvae from larger eggs have increased fitness in cooler climates (Harvey 1983a, 1983b, 1985b; Volney 1985). Therefore, it is surprising that so little evidence for recent hybridization was found between *C. fumiferana* and either western species in this study.

An alternative explanation may be that intrinsic post-zygotic factors may be at play (Coyne and Orr 2004). However, such factors do not appear to contribute to reproductive isolation since hybrids can be acquired through pairings of any pure or hybrid individuals (Campbell 1958, 1967; Sanders *et al*. 1977; Harvey 1997). In addition, although some evidence suggests that there may be rare chromosomal incompatibilities (Smith 1944), their effects on hybrid inviability are not known.

Ecological speciation has been gaining traction in recent years as a significant force in the evolution of species boundaries (Rundle and Whitlock 2001; Rundle and Nosil 2005; Nosil 2012). Strong ecologically-based divergent selection between species adapted to different environments can produce hybrids maladapted to parental environments (Nosil 2012). Here, the current hybrid zone between *C. fumiferana* (boreal) and the western budworm species (montane) is the product of secondary contact that occurred when the distributions of their hosts met in western Canada following the Wisconsinan glaciation (Volney 1985; Powell and DeBenedictis 1995). Thus, dispersal from populations outside the area of contact across the ecological transition zone from boreal to montane habitats may be counter-balanced by strong ecologically-dependent divergent selection on admixed populations giving the hybrid zone characteristics of a tension zone (Barton and Hewitt 1986; Hewitt 1988). In the western group, however, hybridization between *C. biennis* and *C. occidentalis* has likely been occurring since the onset of the Holocene and the deglaciation of the Cordilleran ice sheet (Nealis 2005). The interaction between these western species is more indicative of a “hybrid swarm”, as has been described in

other hybrid systems with post-glacial distributions (Sperling 1987; Latch *et al*. 2011, Haselhorst and Beurkle 2013), with hybrids distributed across most western populations.

The landscape of the hybrid zone is also characterized by a patchy distribution of inhospitable habitats, such as aspen parkland, agricultural cropland, and ranch-land that penetrate the zone of interaction from the east. This may have served to minimize contact between eastern and western species. Recently, difficulties in mate-finding have been shown to induce Allee effects that maintain low-densities of *C. fumiferana* populations below a threshold density (Regniere *et al*. 2012). At species boundaries, particularly in hybrid zones where negative gene-environment interactions are also expected to reduce fitness, this is likely to increase susceptibility to population collapse caused by drift, and to increase rates of inbreeding depression that could impact local adaptation (Holt *et al.* 2004; Bridle and Vines 2007). Therefore, mate-finding Allee effects may represent a form of transient selection against mixed populations occurring in this region that can both stimulate increased rates of gene flow between species during outbreak phases and at other times drive populations to low densities. This could account for long-term gene flow across the hybrid zone between eastern and western species, despite little evidence for recent hybridization. However, the effects of demographic processes on gene flow between species are poorly known (Rasanen and Hendry 2008) and require further investigation.

Limitations

Although the methods employed here were successful in detecting 2277 SNP markers to investigate hybridization and gene flow between these spruce budworm species, it is important to acknowledge several limitations of the data that may influence the results herein, including sampling, library preparation, and marker discovery. The majority of locations were sampled by pheromone trapping and thus there is considerable male-bias within the dataset. Sex-biased dispersal is known to affect gene flow patterns among populations and population structure (Fraser *et al*. 2003). Thus, for species with known sex-biased dispersal, as has been demonstrated in *C. fumiferana* (Greenbank *et al*. 1980), predominantly sampling males may not accurately depicted the genetic relationships between these species. The budworms used in this study also represent a mix of contemporary and historical collections made over the course of the past two decades, although most populations were sampled in the period since 2005. Thus, changes with respect to the landscape, such as fragmentation of natural habitat, may have had some influence on the results (van Dongen *et al*. 1998). This is particularly relevant given that the reference populations used for *C. biennis* contain samples collected primarily from the early 1990s. Also, collections of *C. biennis* may be under-represented in the dataset since many locations were sampled only in a single year. Throughout most of its range *C. biennis* adults emerge on even numbered years, although some populations have been identified where they occur on odd-numbered years, such as in the area of Mr (Shepherd *et al*. 1995; Nealis 2005). However, most of the collections in this dataset were made on even-numbered years, suggesting that this sampling bias may not have a significant effect. Finally, the number of specimens sequenced per population may be low, as population genetic studies typically employ 20-30 individuals per population (Pruett and Winker 2008). However, recent work has demonstrated that the number

and variability of loci has a greater impact on statistical power than the number of individuals (Languth *et al*. 2011). In addition, the numbers of individuals per population used here are comparable to those used in similar hybid zone studies (Haselhorst and Buerkle 2013; Smith *et al*. 2013).

The method used to develop GBS and Radseq libraries is also prone to a wide variety of sources of genotyping error. A thorough list of such sources is compiled in Mastretta-Yanes *et al*. (2014), and includes human error, error associated with wet-lab procedures and sequencing, as well as bioinformatic error. PCR duplication, for instance, is of particular concern as the current double-digest GBS protocol lacks a method of controlling for this factor (Davey *et al*. 2013). PCR duplicates arise when multiple copies of the original DNA molecule anneal to sequencing flowcell oligos resulting in unequal coverage of independent genomic regions represented in the libraries (Davey *et al*. 2013; Mastretta-Yanes *et al*. 2014). This may result in erroneous genotype calls of lower coverage loci since heterozygous genotypes may be falsely identified as homozygous if both alleles are not sequenced. To help overcome PCR duplication issues, stringent filtering was used here to discard loci with greater than 5% missing genotypes, as this should remove most loci with low sequencing coverage. An alternative method could be to use paired-end GBS libraries, since PCR duplicates may be more easily identified using that approach (Davey *et al*. 2013).

Although not a source of error, the use of a desired coverage of 50X per locus per individual in the design of our GBS library may have greatly limited the number of loci available for this work. Sequencing coverage is proportional to the number of sequenced reads divided by the total possible number of tags, which for diploid individuals is equivalent to twice the number of fragments resulting from the restriction enzyme digest. Therefore, the effect of imposing a

greater sequencing coverage is that either fewer individuals or loci can be sequenced at once. Despite this, a substantial number of SNPs (128,241 in total) were found using the approach described in this study, suggesting that high desired coverage was not a significant limitation. Moreover, after filtering for loci with >95% genotype call rates (13,232 SNPs) it was evident that the average read depth across all individuals was closer to 75 reads (see results). In contrast, other studies have opted for a more holistic approach such that more loci are used at the expense of coverage. For example, an average coverage of ~0.63 reads per individual per locus have been used (Parchman *et al*. 2013), and ~0.77 reads per individual per genetic region (Nosil *et al*. 2013). Thus, future work could maximize genomic coverage, and potentially make use of genotype imputation methods to account for missing data, as in Sonah *et al*. (2013).

With regard to marker discovery, the filters imposed in this study greatly reduced the number of loci available for analyses down to just 2277 SNPs. Loci were filtered for genotype coverage, read depth, and those with high linkage disequilibrium within a scaffold were also pruned (see methods). Understandably, the use of such stringent filtering steps may have unnecessarily discarded loci with valuable information content. However, this was done to ensure accuracy in genotype calls as noted above, as well as to reduce the dataset to a number of loci more amenable to standard genetic software, such as Structure (Pritchard *et al*. 2000). In addition, use of the TASSEL-GBS pipeline also results in the loss of a large amount of data as all reads are trimmed to 64 bases. However, this particular functionality of the pipeline is due to be adjusted to allow for longer read lengths in future builds of the software (Glaubitz *et al*. 2014). Alternatively, other openly-available software such as Stacks (Catchen *et al*. 2013) do not impose read trimming and can also compute a number of population genetic statistics. Thus, the use of such tools may be of particular interest for revisiting this data in the immediate future.

Conclusions

Our study highlights the value of modern genomic techniques in identifying complex hybrid dynamics between non-model species. Using GBS techniques, the genetic resources available for the study of spruce budworms have increased substantially. With 2277 high coverage SNPs we have confidently identified the presence and distribution of eastern (*C. fumiferana*), and western (*C. biennis* and *C. occidentalis*) spruce budworms in Alberta. Discrimination of the latter two species, however, was only weakly supported, a finding that is consistent with previously employed molecular approaches (Sperling and Hickey 1994, 1995; Lumley and Sperling 2010, 2011). The degree of genetic similarity and overlap in life history characteristics of these two species, furthermore, casts doubt on their current treatment as separate species. This is also supported by the higher frequency of individuals with admixed ancestry, greater gene flow, and very low genetic differentiation between these two western species relative to that between either of these species and *C. fumiferana.* Contrary to prior hypotheses, patterns of gene flow do not show significant west-to-east asymmetry, although an asymmetric model was best supported. This suggests that our current knowledge of reproductive barriers between spruce budworm species is far from complete. Patterns of hybridization across our study area suggest a balance of gene flow and strong selection against hybrids between eastern and western species that is possibly acting on ecological differences between species, in tandem with demographic effects on the stability of hybridizing populations at species range peripheries. Demonstrating that either of these processes affects gene flow between species will greatly benefit our understanding of interspecific population dynamics and speciation within the spruce budworm species complex, in addition to potentially providing practical insights for forest resource management. Additional work to characterize the locus-specific pattern of

introgression relative to geography and genomic backgrounds should serve to elucidate what kinds of genetic factors contribute to species divergence and the maintenance of species boundaries.

Table 4-1: Population sample sizes and geographic coordinates.

Location		N	Latitude	Longitude	Elev (m)
Beaver Dam Road (AB)	BD	10	N53 56.602	W119 14.656	1466
Beaver Mines (AB)	BM	10	N49 25.091	W114 17.420	1444
Bragg Creek (AB)	BC	12	N50 56.917	W114 34.350	1306
Brightsands Lake (SK)[1]	BL	10 (2)	N53 35.017	W108 54.050	638
Bull River (BC)[2]	BR	15	N49 28.747	W115 27.425	810
Canal Flats (BC)	CF	10	N50 07.005	W115 46.192	839
Casket Lake (AB)	Cs	10 (2)	N53 46.998	W119 55.560	1631
Coleman (AB)	Cm	8	N49 38.617	W114 31.654	1456
Crimson Lake PP (AB)	CL	16 (3)	N52 27.119	W115 1.659	971
Eagle Point PP (AB)	EP	18 (2)	N53 14.880	W114 52.494	764
Edgewater (BC)	Ew	10	N50 46.020	W116 11.734	891
Edmonton (AB)[1]	Ed	14	N53 32.706	W113 26.297	665
Elko (BC)	Ek	5	N49 17.576	W115 09.242	886
Field (BC)	Fd	10 (1)	N51 24.380	W116 28.640	1249
Greenwood (BC)	Gw	10 (1)	N49 05.400	W118 40.650	750
Hailstone Butte (AB)	HB	13	N50 10.461	W114 27.770	1955
Hinton (AB)	Ht	17 (1)	N53 22.925	W 117 41.634	1046
Honeymoon Lake (AB)	HL	10	N52 34.598	W117 43.453	1294
Kalamalka Lake PP (BC)	KL	6 (1)	N50 12.480	W119 16.140	547
Kelowna (BC)	Kw	10	N49 57.668	W119 33.587	693
Kettle Valley RA (BC)	KV	10	N49 06.893	W118 58.813	610
Longview (AB)	Lv	14 (1)	N50 28.622	W114 25.407	1351
Maycroft (AB)	Mc	11	N49 50.751	W114 17.059	1451
McBride (BC)[3]	Mb	11	N53 18.000	W120 10.000	726
Merritt (BC)	Mt	9	N50 6.678	W120 47.173	600
Moberly Tower Rd (AB)	My	10 (2)	N53 32.142	W118 01.742	1579
Monte Creek (BC)	Mn	5	N50 39.000	W119 57.000	356
Morrisey Creek (BC)[3]	Mr	5	N56 19.998	W124 33.000	1261
Mount Shark (AB)	MS	10 (1)	N50 35.244	W115 21.720	2214
Nordegg (AB)	Nd	12 (3)	N52 28.700	W116 06.659	1346
Numa Falls (BC)	NF	11	N51 07.865	W116 07.581	1411
Pembina Forks (AB)	PF	14	N52 58.033	W116 36.838	1317
Pembina River PP (AB)	PR	17	N53 36.357	W115 00.273	725

Reference populations for *Choristoneura fumiferana*[1], *Choristoneura occidentalis*[2], and *Choristoneura biennis*[3] were chosen on the basis of geographic separation and associated host information. Sample sizes (N) in parentheses indicate the number of individuals discarded from the analysis on the basis of low sequencing coverage.

Table 4-1: (continued)

Location		N	Latitude	Longitude	Elev (m)
Pocaterra RA (AB)	Pc	5	N50 41.322	W115 06.372	1642
Porcupine Hills - North (AB)	P1	17	N49 52.007	W114 00.466	1592
Porcupine Hills – South (AB)[2]	P2	11	N49 48.573	W113 57.307	1410
Ram Falls PP (AB)	RF	13	N52 05.208	W115 52.781	1639
Red Lodge PP (AB)	RL	12	N51 56.683	W114 14.290	954
Saskatchewan River Crossing (AB)	SX	10	N51 58.378	W116 44.097	1457
Sibbald (AB)	Sb	10	N51 03.139	W114 56.547	1554
Smith Lake (AB)	SL	5	N51 15.658	W115 56.009	1441
Sparwood (BC)	Sw	10 (1)	N49 43.557	W114 51.100	1248
Strawberry Ridge (AB)	SR	10	N51 46.566	W115 14.886	1426
Wolf Lake Road (AB)	WL	10 (4)	N53 29.488	W116 04.054	962

Reference populations for *Choristoneura fumiferana*[1], *Choristoneura occidentalis*[2], and *Choristoneura biennis*[3] were chosen on the basis of geographic separation and associated host information. Sample sizes (N) in parentheses indicate the number of individuals discarded from the analysis on the basis of low sequencing coverage.

Table 4-2: Mean samples size (N), observed (H_o) and unbiased expected heterozygosity (uH_e), and fixation index (F) across 2277 loci for each population.

Population	N	H_o	uH_e	F
BD	9.976	0.232	0.206	-0.123
BM	9.960	0.207	0.195	-0.074
BC	11.950	0.196	0.237	0.091
BL[1]	7.933	0.184	0.178	-0.084
BR[2]	14.953	0.198	0.189	-0.039
CF	9.968	0.218	0.202	-0.091
Cs	7.962	0.209	0.197	-0.098
Cm	7.964	0.203	0.191	-0.102
CL	12.934	0.192	0.238	0.092
EP	15.897	0.178	0.188	0.076
Ew	9.977	0.219	0.203	-0.088
Ed[1]	13.910	0.173	0.169	-0.033
Ek	4.988	0.193	0.188	-0.124
Fd	8.947	0.198	0.185	-0.094
Gw	8.971	0.199	0.189	-0.074
HB	12.944	0.204	0.221	0.070
Ht	15.930	0.196	0.188	-0.034
HL	9.956	0.214	0.195	-0.098
KL	4.979	0.188	0.183	-0.120
Kw	9.968	0.213	0.193	-0.103
KV	9.974	0.212	0.195	-0.097
Lv	12.944	0.195	0.241	0.092
Mc	10.949	0.196	0.185	-0.067
Mb[3]	10.951	0.204	0.196	-0.056
Mt	8.949	0.189	0.191	-0.025
My	7.932	0.215	0.196	-0.119
Mn	4.975	0.191	0.190	-0.101
Mr[3]	4.981	0.216	0.205	-0.144
MS	8.982	0.225	0.202	-0.119
Nd	8.958	0.193	0.226	0.077

Reference populations for *Choristoneura fumiferana*[1], *Choristoneura occidentalis*[2], and *Choristoneura biennis*[3] were chosen on the basis of geographic separation and associated host information.

Table 4-2: (continued)

Population	N	H_o	uH_e	F
NF	10.946	0.200	0.194	-0.044
PF	13.949	0.193	0.215	0.091
PR	16.902	0.179	0.187	0.066
Pc	9.942	0.193	0.185	-0.067
P1	4.964	0.198	0.199	-0.092
P2[2]	16.934	0.200	0.187	-0.055
RF	10.949	0.215	0.197	-0.095
RL	12.941	0.204	0.192	-0.067
SX	11.936	0.184	0.212	0.092
Sb	9.916	0.195	0.184	-0.080
SL	9.975	0.216	0.198	-0.101
Sw	4.970	0.183	0.175	-0.139
SR	8.959	0.192	0.183	-0.078
WL	9.965	0.205	0.198	-0.059
Total	5.970	0.269	0.249	-0.142

Reference populations for *Choristoneura fumiferana*[1], *Choristoneura occidentalis*[2], and *Choristoneura biennis*[3] were chosen on the basis of geographic separation and associated host information.

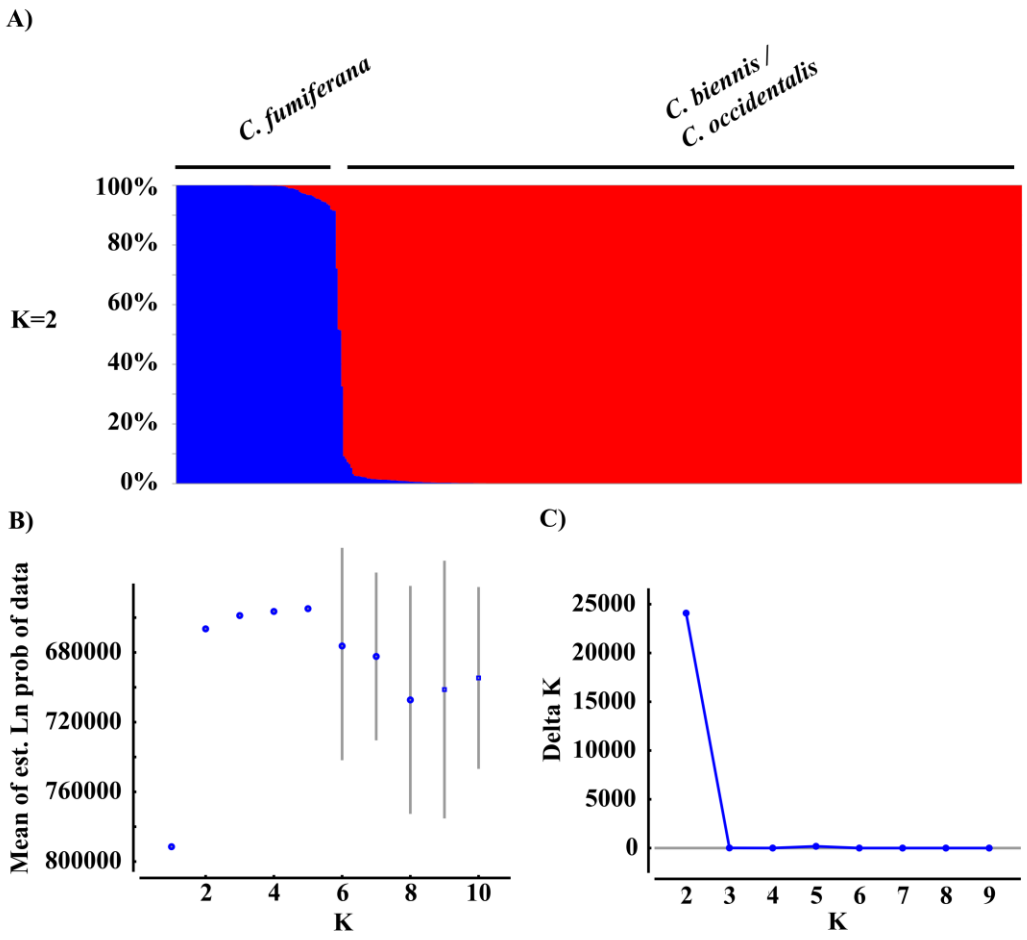


Figure 4-1. Structure results showing A) barplot for individuals assigned to *K*=2 genetic clusters; B) distribution of mean estimates of the log probability of the data at each value of *K*; and C) delta *K* plot resulting from the Evanno *et al*. (2005) method. Individuals in A) are sorted by their ancestry assignment to each cluster at *K*=2. Results present in B and C show significant support for *K=2* over larger values of *K*.

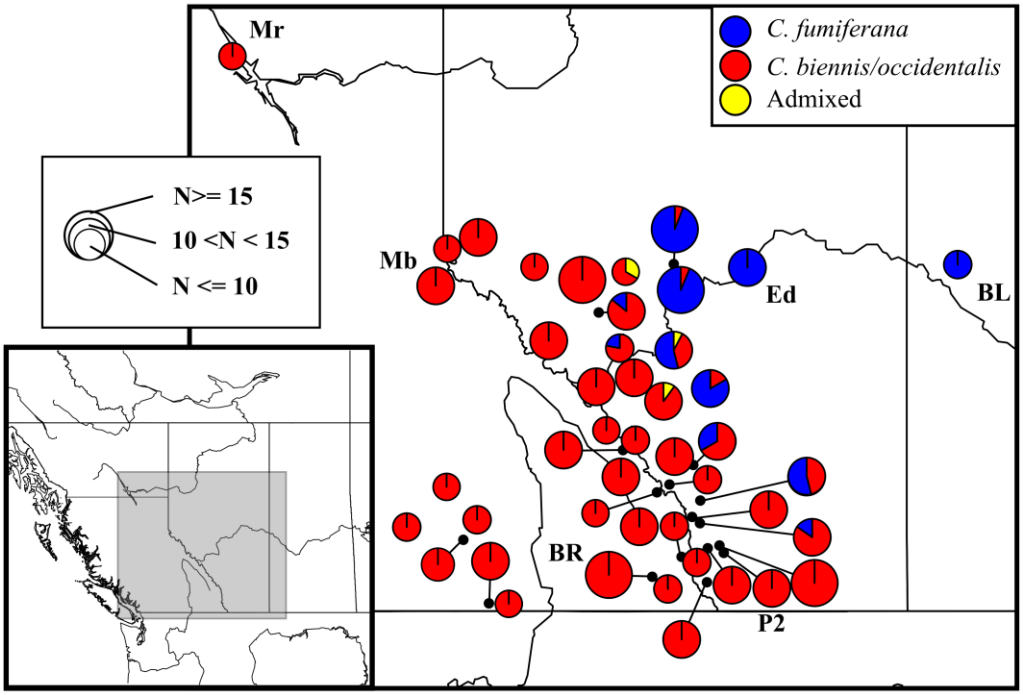


Figure 4-2. Geographic distribution of sampled populations showing the populations with admixed individuals between eastern and western spruce budworm genetic clusters. Populations are represented as pie charts indicating the proportion of each population with ancestry assignment (q >= 0.9) to each genetic cluster identified by Structure at K=2. Clusters correspond with *C. fumiferana,* and a western cluster consisting of *C. biennis* and *C. occidentalis*. Pie chart size corresponds with sample size (N) at each location. Reference populations for *C. fumiferana*, *C. occidentalis* and *C. biennis* are labeled next to their corresponding pie charts. The inset shows the location of the sampled area relative to western Canada.

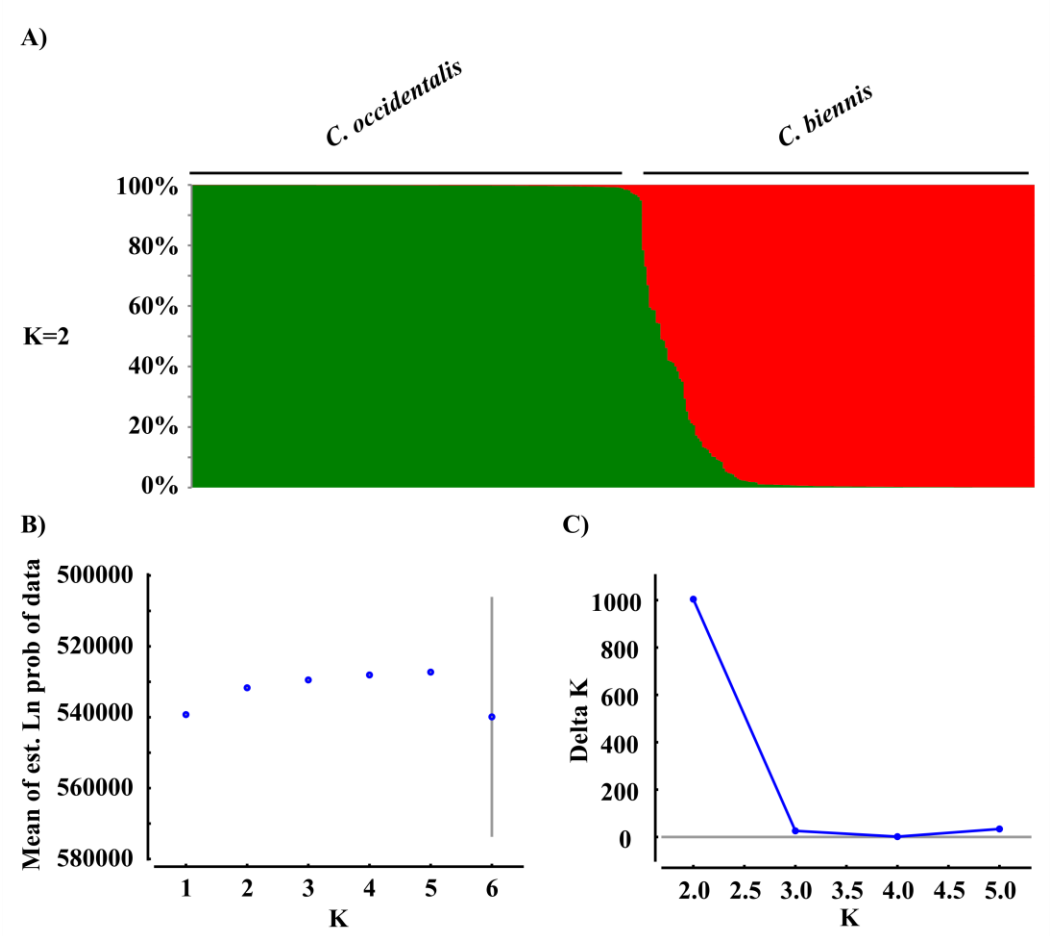


Figure 4-3. Structure results for the hierarchical analysis of substructure within the *K*=2 western genetic cluster consisting of 365 individuals with >95% assignment probability. A) shows the barplot for western individuals assigned to *K*=2 subpopulations; B) the distribution of mean estimates of the log probability of the data at each value of *K*; and C) the delta *K* plot resulting from the Evanno *et al*. (2005) method. Individuals in A) are sorted by their ancestry assignment to each western subpopulation at *K*=2. Results present in B and C show weak support for *K*=2.

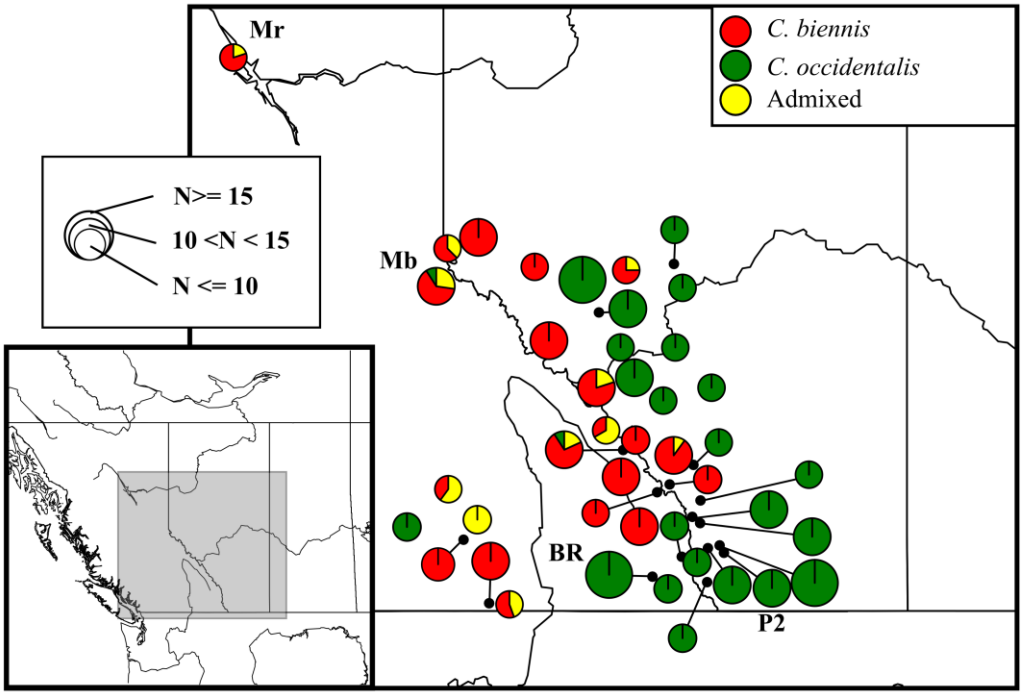


Figure 4-4. Geographic distribution of population substructure within the western genetic cluster showing the populations with admixed individuals between the western subpopulations. Populations are represented as pie charts indicating the proportion of each population with ancestry assignment (q >= 0.9) to each of the western subpopulations identified by Structure at K=2 when only western individuals are considered (N=365). The subpopulations correspond with *C. occidentalis* and *C. biennis* reference populations, respectively. Pie chart size corresponds with sample size (N) at each location. Reference populations for *C. occidentalis* and *C. biennis* are labeled next to their corresponding pie charts. The inset shows the location of the sampled area relative to western Canada.

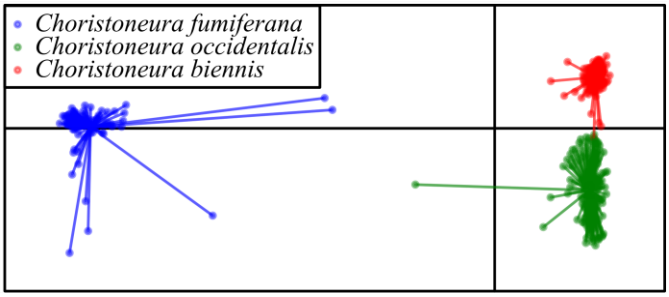


Figure 4-5. Discriminant analysis of principal components (DAPC) scatterplot showing the clustering of individuals along the first two principal components. Individuals cluster into three groups that correspond with *Choristoneura fumiferana*, *C. occidentalis*, and *C. biennis* as determined based on clustering with reference populations of each species.

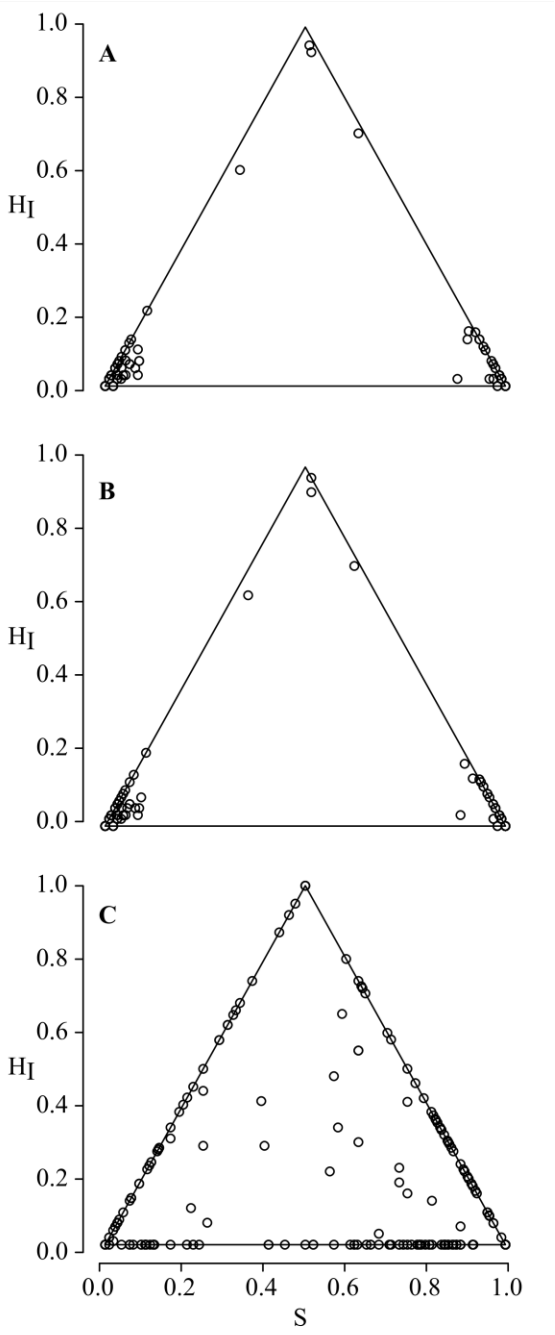


Figure 4-6. Triangle plots resulting from two-way hybrid analysis in HIest. Plots show the proportion of markers heterozygous (H_I) for *Choristoneura fumiferana* and *C. occidentalis* alleles (A), *C. fumiferana* and *C. biennis* alleles (B), and *C. occidentalis* and *C. biennis* alleles (C) with each individual plotted against hybrid index (S).

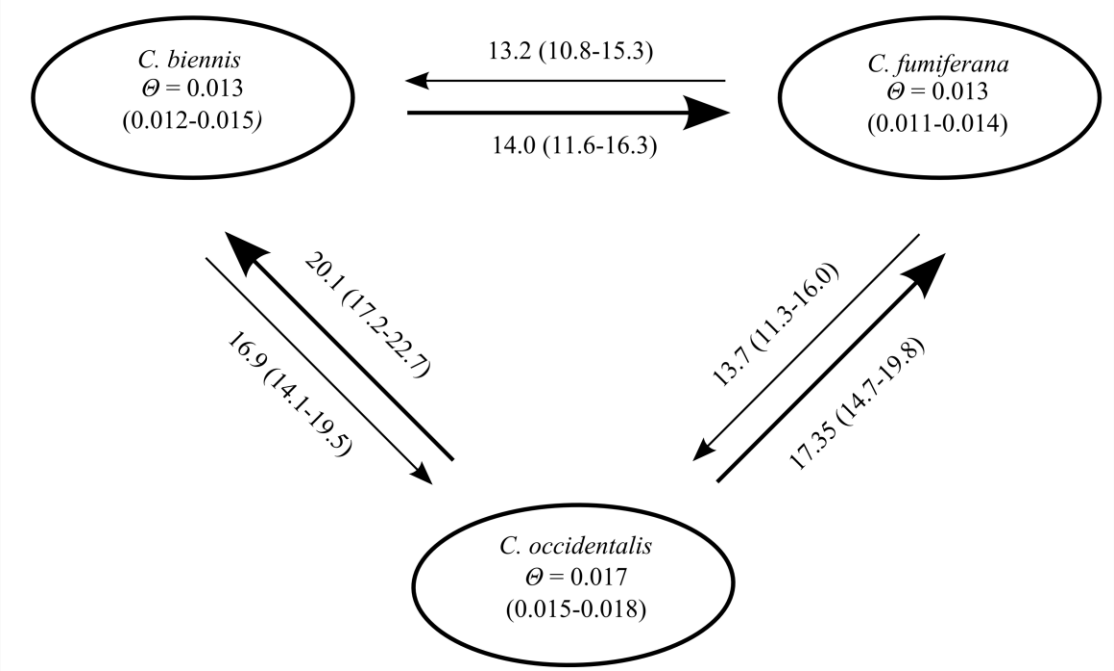


Figure 4-7. Asymmetric gene flow model resulting from Migrate-n analyses showing modal estimates (with 95% support limits) of mutation-scaled effective population size (Θ) for *C.fumiferana, C. biennis*, and *C. occidentalis*, as well as the effective number of migrants per generation (N_em, above and below arrows) in each direction between the three species.

Literature Cited

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Most, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice., A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., stelkens, R., Szymura, J.M., Vainola, R., Wolf, J.B.W., and D. Zinner. 2012. Hybridization and speciation. Journal of Evolutionary Biology, 26: 229-246.

Anderson, E. 1948. Hybridization of the habitat. Evolution, 2: 1-9.

Anderson, E.C., and E.A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics, 160: 1217-1229.

Barton, N.H., and G.M. Hewitt. 1985 Analysis of hybrid zones. Annual Review of Ecology and Systematics, 16: 113-148.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics, 22: 341-345.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy Sciences of the United States of America, 98: 4563-4568.

Beerli, P., M. Palczewski. 2010. Unified framwork to evaluate panmixa and migration direction among mulitple sampling locations. Genetics, 185: 313-326.

Bird, H.M. 2013. Phylogenomics of the *Choristoneura* fumiferana species complex (Lepidopter: Tortricidae). MSc Thesis, University of Alberta, Edmonton, Canada.

Bridle, J.R., and T.H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends in Ecology and Evolution, 22: 140-147.

Brunet, B.T.M., Hundsdoerfer, A.E., and F.A.H. Sperling. 2013. Identification and ecological characterisation of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada. The Canadian Entomologist, 145: 521-528.

Buerkle, C.A., Morris, R.J., Asmussens, M.A., and L.H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. Heredity, 84: 441-451.

Buerkle, C.A. 2005. Maximum-likelihood estimation of a hybrid index based on molecular markers. Molecular Ecology Notes, 5: 684-687.

Butlin, R. 1987 Speciation by reinforcement. Trends in Ecology and Evolution, 2: 8-13.

Campbell, I.M. 1958. A genetic study of factors determining fecundity in the genus *Choristoneura* Lederer (Clem.) (Tortricidae). PhD Thesis, University of Toronto, Toronto, Canada.

Campbell, I.M. 1962. Reproductive capacity in the genus Choristoneura Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers or rates. Canadian Journal of Genetics and Cytology, 4: 272-288.

Campbell, I.M. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist, 99: 482-486.

Catchen, J.M., Hohenlohe, P.A., Bassham, S., Amores, A., and W.A., Cresko. 2013, Stacks: an analysis tool set for population genomics. Molecular Ecology, 22: 3124-3140.

Chenuil, A. 2012. How to infer reliable diploid genotypes from NGS or traditional sequence data: from basic probability to experimental optimization. Journal of Evolutionary Biology, 25: 949-960.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinuaer Associates, Inc., Sunderland, Massachusetts. Pp. 543.

Currat, M., Ruedi, M., Petit, R.J., and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution, 62: 1908-1920.

Danecek, P., Auton, A., Abecasis, G., Albers, C., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics, 27: 2156-2158.

Davey, J.W., and M.L. Blaxter. 2010. RADSeq: next-generation population genetics. Briefings in Functional Genomics, 9: 416-423.

Davey, J.W., Cezard, T., Fuentes-Utrilla, P., Eland, C., Gharbi, K., and M.L. Blaxter. 2013. Special features of RAD sequening data: implications for genotyping. Molecular Ecology, 22: 3151-3164.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., and M.L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12: 499-510.

Deverno, L.L., Smith,. G.A., and K.J. Harrison. 1998. Randomly amplified polymorphic DNA evidence of introgression in two closely related sympatric species of coniferophagous Choristoneura (Lepidoptera: Tortricidae) in Atlantic Canada. Annals of the Entomological Society of America, 91: 248-259.

Earl, D.A., and B.M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetic Resources, 4: 359-361.

Evanno, G., Regnaut, S., and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14: 2611-2620.

Falush, D., Stephens, M., and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164: 1567-1587.

Fitzpatrick, B.M. 2012. Estimating ancestry and heterozygosity of hybrids using molecular markers. BMC Evolutionary Biology, 12: 131. https://www.biomedcentral.com/1471-2148/12/131

Fraser, D.J., Lippe, C., and L. Bernatchez. 2003. Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Molecular Ecology, 13: 67-80.

Freeman, T.N. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. I. Some new forms of *Choristoneura* allied to *C. fumiferana*. The Canadian Entomologist, 99: 449-455.

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q., and E.S. Buckler. 2014. TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS ONE 9(2): e90346. Doi:10.1371/journal.pone.0090346.

Gray, T.G., Shepherd, R.F., and G.T. Harvey. 1995. Incidence of spicules on the aedeagi of *Choristoneura fumiferana*, *C. biennis,* and *C.*unidentified species (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 161-166.

Greenbank, D.O., Schaefer, G.W., and R.C. Rainey. 1980. Spruce budworm (Lepidoptera: Tortricidae) moth flight and dispersal: new understanding from canopy observations, radar, and aircraft. Memoirs of the Entomological Society of Canada, No. 110. pp. 49.

Haselhorst, M.S.H., and C.A. Buerkle. 2013. Population genetic structure of Picea engelmannii, P. glauca and their previously unrecognized hybrids in the central Rocky Mountains. Tree Genetic & Genomes, 9: 669-681.

Harvey, G.T. 1961. Second Diapause in spruce budworm from eastern Canada. The Canadian Entomologist, 93: 594-602.

Harvey, G.T. 1983a. A geographical cline in egg weights in *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and its significance in population dynamics. The Canadian Entomologist, 115: 1103-1108.

Harvey, G.T. 1983b. Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 115: 1109-1117.

Harvey, G.T. 1985a. The taxonomy of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): A review. Pp. 16-59 in C.J. Sanders, R.W. Stark, E.J. Mullins, And J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Harvey, G.T. 1985b. Egg weight as a factor in the overwintering survival of spruce budworm (Lepidoptera: Tortricidae) larvae. The Canadian Entomologist, 117: 1451-1461.

Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferphagous *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519-536.

Hewitt, G.M. 1988. Hybrid zones - Natural Laboratories for evolutionary studies. Trends in Ecology and Evolution, 3: 158-167.

Hohenlohe, P.A., Amish, S.J., Catchen, J.M., Allendorf, F.W., and G. Luikart. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources, 11: 117-122.

Holt, R.D., Knight, T.M., and M. Barfield. 2004. Allee effects, immigration, and the evolution of species' niches. The American Naturalist, 163: 253-262.

Jakobsson, M. and N.A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23: 1801-1806.

Jiggins, C.D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in Ecology and Evolution, 15: 250-255.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structure populations. BMC Genetics, 11: 94. http://www.biomedcentral.com/1471-2156/11/94

Languth, E.L., Fedy, B.C., Oyler-McCance, S.J., Garey, A.L., Emel, S.L., Mumma, M., Wagner, H.H., Fortin, M.-J., and S.A. Cushman. 2011. Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. Molecular Ecology Resources, 12: 276-284.

Latch, E.K., Heffelfinger, J.R., Fike, J., and O.E., Rhodes. 2009. Species-wide phylogeography of North American mule deer (Odocoileus hemionus): cryptic glacial refugia postglacial recolonization. Molecular Ecology, 18: 1730-1745.

Lexer, C., Kremer, A., and R.J. Petit. 2006. Shared alleles in sympatric oaks: recurrent gene flow is a more parsimonius explanation than ancestral polymorphism. Molecular Ecology, 15: 2007-2012.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25: 1754-1760.

Liebhold, A.M., Volney, W.J.A., and W.E. Waters. 1984. Evaluation of cross-attraction between sympatric *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 827-840.

Lumley, L.M. 2010. Species delimitation in the *Choristoneura fumiferana* species complex (Lepidoptera: Tortricidae). Ph. D. Thesis. University of Alberta, Edmonton, Canada.

Lumley, L.M., and F.A.H. Sperling. 2011a. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232-243.

Lumley, L.M., and F.A.H. Sperling. 2011b. Life-history traits maintin the genomic integrity of sympatric species of the spruce budworm (Choristoneura fumiferana) group on an isolated forest island). Ecology and Evolution, 1: 119-131.

Mallet, J. 1995. A species definition for the Modern Synthesis. Trends in Ecology and Evolution, 10: 294-299.

Mallet, J. 2007. Hybrid speciation. Nature, 446: 279-283.

Martin, N.H., Bouck, A.C., and M.L. Arnold. 2006. Detecting adaptive trait introgression between Iris fulva and I. brevicaulis in highly selective field conditions. Genetics, 172: 2481-2489.

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Pinero, D., and B.C. Emerson. 2014. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for populations genetic inference. Molecular Ecology resources, doi:10.111/1755-0998.12291.

Mayr, E. 1969. The biological meaning of species. Biological Journal of the Linnean Society, 1: 311-320.

Minder, A.M., and A. Widmer. 2008. A population genomic analysis of species boundaries: neutral processes, adaptive divergence and introgression between two hybridizing plant species. Molecular Ecology, 17: 1552-1563.

Morjan, C.L., and L.H. Rieseberg. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Molecular Ecology, 13: 1341-1356.

Nealis, V.G. 2005. Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist, 137: 584-597.

Nolte, A.W., and D. Tautz. 2010. Understanding the onset of hybrid speciation. Trends in Genetics, 26: 54-58.

Nosil, P. 2012. Ecological Speciation. Oxford University Press, University of Oxford, Oxford, United Kindom. 280pp.

Nosil, P., Gompert, Z., Farkas, T.E., Comeault, A.A., Feder, J.L., Buerkle, C.A., and T.L. Parchman. 2013. Genomic consequences of multiple speciation processes in a stick insect. Proceedings of the Royal Society of London B, 279: 5058-5065.

Parchman, T.L., Gompert, Z., Braun, M.J., Brumfield, R.T., McDonald, D.B., Uy, J.A.C., Jarvis, E.D., Schlinger, B.A., and C.A. Buerkle. 2013. The genomic consequences of adaptive divergence and reproductive divergence and reproductive isolation between species of manakins. Molecular Ecology, 22: 3304-3317.

Parsons, T.J., Olson, S.L., and M.J. Braun. 2006. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. Science, 260: 1643-1646.

Peakall, r., and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Populations genetic software for teaching and research-an update. Bioinformatics, 28: 2537-2539.

Petit, R.J., and L. Excoffier. 2009. Gene flow and species delimitation. *Trends in Ecology and Evolution*, 24: 386-393.

Poland, J.A., Brown, P.J., Sorrells, M.E., and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLOS One, 7:e32253. doi:10.1371/journal.pone.0032253.

Powell, J.A., and J.A. DeBenedictis. 1995. Evolutionary interpretations, taxonomy, and nomenclature. Pp. 217-275 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Pritchard, J.K., Stephens, M., and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Pruett, C.L., and K. Winker. 2008. The effects of sample size on populations genetic diversity estimates in song sparrows *Melospiza melodia*. Journal of Avian Biology, 39: 252-256.

Rasanen, K., and A.P. Hendry. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. Ecology Letters, 11: 624-636.

Razowski, J. 2008. Tortricidae (Lepidoptera) from South Africa. 6: *Choristoneura* Hubner and Procrica Diakonoff. Polish Journal of Entomology, 77: 245-254.

Regniere, J., Delisle, J., Pureswaran, D.S., and R. Trudel. 2012. Mate-finding allee effect in spruce budworm ppulation dynamics. Entomologia Experimentalis et Applicata, 146: 112-122.

Rieseberg, L.H., Atrcher, M.A., and R.K. Wayne. Transgressive segregation, adaptation and speciation. Heredity, 83: 363-372.

Rundle, H.D., and M.C. Whitlock. 2001. A genetic interpretation of ecologically dependent isolation. Evolution, 55: 198-201.

Rundle, H.D., and P. Nosil. Ecological speciation. Ecology Letters, 8: 336-352.

Sanders, C.J. 1971a. Sex pheromone specificity and taxonomy of budworm moths (*Choristoneura*). Science, 171: 911-913.

Sanders, C.J. 1971b. Daily activity patterns and sex pheromone specificty as sexual isolating mechanisms in two species of *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 103: 498-502.

Sanders, C.J., Daterman, G.E., and T.J. Ennis. 1977. Sex pheromone responses of *Choristoneura* spp. and their hybrids (Lepidoptera: Tortricidae). The Canadian entomologist, 109: 1203-1220.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution, 19: 198-207.

Servedio, M.R., and M.A.F. Noor. 2003. The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution, and Systematics, 34: 339-364.

Shepherd, R.F., Gray, T.G., and G.T. Harvey. 1995. Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) fedding on Abies, Picea, and Pseudotsuga in western Canada and Alaska. The Canadian Entomologist, 127: 813-830.

Smith, K.L., Hale, J.M., Kearney, M.R., Austin, J.J., and J. Melville. 2013. Molecular patterns of introgression in a classic hybrid zone between the Australian tree fogs, Litoria ewingii and L. paraewingi: evidence of a tension zone. Molecular Ecology, 22: 1869-1883.

Smith, S.G. 1944. The reproduction of the nucleus. Scientific Agriculture, 25: 566-571.

Smith, S.G. 1953. Reproductive isolation and the integrity of two sympatric species of *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 85: 141-151.

Smith, S.G. 1954. A partial breakdown of temporal and ecological isolation between *Choristoneura* species (Lepidoptera: Tortricidae). Evolution, 8: 206-224.

Sonah, H., Bastien, M., Iquira, E., Tardive, A., Legare, G., Boyle, B., Normandeau, E., Laroche, J., Larose, S., Jean, M., and F. Belzile. 2013. An imporved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PLOS One, 8:e54603. doi:10.1371/journal.pone.0054603

Sperling, F.A.H. 2003. Butterfly Molecular Systematics: from species defintions to higher-level phylogenies. Pp. 431-458 in C.L. Boggs, W.B. Watt, and P.R. Ehrlich (Eds.), Butterflies: Ecology and Evolution taking flight. University of Chicago Press.

Sperling, F.A.H., and D.A. Hickey. 1994. Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). Molecular Biology and Evolution, 11: 656-665.

Sperling, F.A.H., and D.A. Hickey. 1995. Amplified mitochondrial DNA as a diagnostic marker for species of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 277-288.

Stehr, G.W. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. II. Geographic distribution in accordance with forest regions. The Canadian Entomologist, 99: 456-463.

Stolting, K.N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. , and C. Lexer. 2013. Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. Molecular Ecology, 22: 842-855.

Van Dongen, S., Backeljau, T., Matthysen, E., and A.A. Dhondt. 1998. Genetic population structure of the winter moth (*Operophtera brumata* L.) (Lepidoptera, Geometridiae) in a fragmented landscape). Heredity, 80: 92-100.

Volney, W.J.A, Waters, W.E., Akers, R.P., and A.M. Liebhold. 1983. Variation in spring emergence patterns among western *Choristoneura* spp. (Lepidoptera: Tortricidae) populations in southern Oregon. The Canadian Entomologist, 115: 199-209.

Volney, W.J.A. 1985. Comparative population biologies of North American spruce budworms. Pp. 71-84 in C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Volney, W.J.A., and A.M. Liebhold. 1985. Post-diapause development of sympatric *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) and their hybrids. The Canadian Entomologist, 117: 1479-1488.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characterisitics. Global Change Biology, 13: 1630-1643.

Volney, W.J.A., Liebhold, A.M., and W.E. Waters. 1984. Host associations, phenotypic variation, and mating compatibility of *Choristoneura occidentalis* and *C. retiniana* (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 813-826.

Wu, C.-I. 2001. The genic view of the process of speciation. Journal of Evolutionary Biology, 14: 851-865.

Zhulidov, P.A., Bogdanova, E.A., Shcheglov, A.S., Vagner, L.L., Khaspekov, G.L., Kozhemyako, V.B., Matz, M.V., Meleshkevitch, E., Moroz, L.L., Lukyanov, S.A., and D.A. Shagin. 2004. Simple cDNA normalization using kamchatka crab duplex-specific nuclease. Nucleic Acids Research, 32:e37. doi:10.1093/nar/gnh031.

Appendix 4-1: Specimen GBS barcode sequence and collection data.

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
8923	ACCTAA	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8925	GTACTT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8927	GAATTCA	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8929	ATTGGAT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8930	TCTGTGA	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8932	CGCGATAT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8935	ACCGT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8937	GCTGTGGA	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8938	AACT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
8939	CGCTT	Beaver Dam Road	AB	C. Charbonneau	24/09/2012	15/06/2012	N53 56.60220	W119 14.65620	1466	WS	Unitrap	Male	Y
7873	GTTGAA	Beaver Mines	AB	B. Brunet	21/07/2009	30/07/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7874	GAACTTG	Beaver Mines	AB	B. Brunet	21/07/2009	30/07/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7875	CATAAGT	Beaver Mines	AB	B. Brunet	21/07/2009	30/07/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7876	TGCTGGA	Beaver Mines	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7878	CGCCTTAT	Beaver Mines	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7879	GGATTGGT	Beaver Mines	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7880	GCGT	Beaver Mines	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7882	TCACG	Beaver Mines	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7884	GCTTA	Beaver Mines	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7891	GTCAA	Beaver Mines	AB	B. Brunet	12/09/2009	21/09/2009	N49 25.091	W114 17.420	1444	WS	Unitrap	Male	Y
7236	GCTTA	Bragg Creek	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7241	GTCAA	Bragg Creek	AB	B. Brunet	31/07/2010	20/08/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7242	GCTCTA	Bragg Creek	AB	B. Brunet	31/07/2010	20/08/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7243	ATCGTA	Bragg Creek	AB	B. Brunet	31/07/2010	20/08/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7244	TAACGA	Bragg Creek	AB	B. Brunet	31/07/2010	20/08/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7245	GGACCTA	Bragg Creek	AB	B. Brunet	31/07/2010	20/08/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7247	CGCTGAT	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7248	ACGACTAG	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7249	AACCGAGA	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7250	GTGAGGGT	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7251	CGAT	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
7252	CTAGG	Bragg Creek	AB	B. Brunet, S. Brunet	20/08/2010	05/09/2010	N50 56.917	W114 34.350	1306	WS	Unitrap	Male	Y
8037	GGATTGGT	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	Unitrap	Male	Y
8038	GTGAGGGT	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	Unitrap	Male	Y
8039	TATCGGGA	Brightsands Lake	SK	B. Brunet, J.Dupuis	05/07/2012	06/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	Y
8040	TAGCATGG	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	Unitrap	Male	Y
8041	ACAGGGA	Brightsands Lake	SK	B. Brunet, J.Dupuis	05/07/2012	06/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	Unitrap	Male	Y
8042	TTCCTGGA	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	Y
8043	CTCG	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	N
8045	TATCGGGA	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	Y
8047	TGCA	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	Y
8076	ACCGT	Brightsands Lake	SK	B. Brunet, J.Dupuis	06/07/2012	07/07/2012	N53 35.0166	W108 54.0498	638	WS & JP	UV	Male	N
7001	CTCG	Bull River	BC	B. Brunet	20/06/2010	11/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7002	AGCG	Bull River	BC	B. Brunet	20/06/2010	11/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7009	TTCTG	Bull River	BC	B. Brunet	31/07/2010	21/08/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7011	ATTGA	Bull River	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7043	GCCAGT	Bull River	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample passed GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep.t Date	Coll Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7044	ATGAAAG	Bull River	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7045	ACGTGTT	Bull River	BC	B. Brunet	31/07/2010	21/08/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7046	TAGCGGA	Bull River	BC	B. Brunet	31/07/2010	21/08/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7047	TGGTACGT	Bull River	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7049	CGCGGAGA	Bull River	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7050	ACTA	Bull River	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7051	TCAG	Bull River	BC	B. Brunet	31/07/2010	21/08/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7052	GTATT	Bull River	BC	B. Brunet	31/07/2010	21/08/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7053	CCTAG	Bull River	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7054	CCACGT	Bull River	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 28.747	W115 27.425	810	DF & PP	Unitrap	Male	Y
7527	TAATA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7528	CCACAA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7529	CATCGT	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7531	TGGCTA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7535	GTCGATT	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7537	CGGTAGA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7538	TAGCATGG	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7539	ACAGGGA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7540	TATCGGGA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
7541	GTAA	Canal Flats	BC	L. Lumley	29/06/2008	17/08/2008	N50 07.005	W115 46.192	839	DF, PP, LP & LA	Unitrap	Male	Y
9582	CGCGGT	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	N
9583	TATTTTT	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9584	AACGCCT	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9585	CTACGGA	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9586	TAGGCCAT	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	N
9587	ACGTGGTA	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9588	TTCCTGGA	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9589	AGCG	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9590	TTCTG	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
9591	ATTGA	Casket Lake	AB	E. Lee	05/07/2005	05/11/2005	N53 46.998	W119 55.56	1631	WS	Unitrap	Male	Y
7894	GCTCTA	Coleman	AB	B. Brunet	12/07/2009	21/07/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7897	ATCGTA	Coleman	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7899	TAACGA	Coleman	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7901	GGACCTA	Coleman	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7902	CGCTGAT	Coleman	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7904	ACGACTAG	Coleman	AB	B. Brunet	17/08/2009	26/08/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7908	AACCGAGA	Coleman	AB	A. Hundsdoerfer	03/09/2009	12/09/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7910	GTGAGGGT	Coleman	AB	B. Brunet	12/09/2009	21/09/2009	N49 38.617	W114 31.654	1456	DF	Unitrap	Male	Y
7133	TTCCTGGA	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7134	CTCG	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7135	AGCG	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7136	TTCTG	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7137	ATTGA	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7138	TCGTT	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7139	GAGATA	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7140	CTATTA	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	N
7141	CTTGCTT	Crimson Lake PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7144	AATATGG	Crimson Lake PP	AB	B. Brunet	01/08/2010	21/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7145	GCGGAAT	Crimson Lake PP	AB	B. Brunet	01/08/2010	21/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7146	TGCAAGGA	Crimson Lake PP	AB	B. Brunet	01/08/2010	21/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	N
7147	CCATGGGT	Crimson Lake PP	AB	B. Brunet	01/08/2010	21/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	N
7148	TGCA	Crimson Lake PP	AB	B. Brunet	01/08/2010	21/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7151	GATG	Crimson Lake PP	AB	B. Brunet	14/07/2010	01/08/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7152	AGCCG	Crimson Lake PP	AB	B. Brunet, S. Brunet	21/08/2010	04/09/2010	N52 27.119	W115 1.659	971	WS	Unitrap	Male	Y
7113	CATCGT	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7114	TGGCTA	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7115	GTCGATT	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7116	CGGTAGA	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	N
7117	TAGCATGG	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7118	ACAGGGA	Eagle Point PP	AB	B. Brunet	21/06/2010	14/07/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7119	TATCGGGA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7120	GTAA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7121	ACAAA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7122	AGGAT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7123	TACAT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7124	CTTCCA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7126	CGCGGT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	N
7127	TATTTTT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7129	AACGCCT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7130	CTACGGA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7131	TAGGCCAT	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7132	ACGTGGTA	Eagle Point PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N53 14.880	W114 52.494	764	WS	Unitrap	Male	Y
7515	TAACGA	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7516	GGACCTA	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7517	CGCTGAT	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7520	ACGACTAG	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7521	AACCGAGA	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7522	ATTGGAT	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7523	GTGAGGGT	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7524	CGAT	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7525	CTAGG	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7526	GGTGT	Edgewater	BC	L. Lumley	12/07/2006	24/07/2006	N50 46.02	W116 11.734	891	DF	Unitrap	Male	Y
7014	TCGTT	Edmonton	AB	G. Anweiller	29/06/2010	03/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7017	GAGATA	Edmonton	AB	G. Anweiller	06/07/2010	15/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7030	CTATTA	Edmonton	AB	G. Anweiller	15/07/2010	21/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7031	CTTGCTT	Edmonton	AB	G. Anweiller	29/06/2010	03/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7032	AATATGG	Edmonton	AB	G. Anweiller	29/06/2010	30/06/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7033	GCGGAAT	Edmonton	AB	G. Anweiller	06/07/2010	15/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7034	TGCAAGGA	Edmonton	AB	G. Anweiller	15/07/2010	24/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7035	CCATGGGT	Edmonton	AB	G. Anweiller	28/06/2010	05/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7036	TGCA	Edmonton	AB	G. Anweiller	15/07/2010	21/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7037	GATG	Edmonton	AB	G. Anweiller	29/06/2010	03/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7038	AGCCG	Edmonton	AB	G. Anweiller	29/06/2010	30/06/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7039	CATCT	Edmonton	AB	G. Anweiller	06/07/2010	15/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7041	GGTTGT	Edmonton	AB	G. Anweiller	28/06/2010	05/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7042	ATGCCT	Edmonton	AB	G. Anweiller	15/07/2010	21/07/2010	N53 32.706	W113 26.297	665	BS & WS	Unitrap	Male	Y
7940	ACAGGGA	Elko	BC	B. Brunet	31/07/2010	21/08/2010	N49 17.576	W115 09.242	886	DF & PP	Unitrap	Male	Y
7941	TATCGGGA	Elko	BC	B. Brunet	31/07/2010	21/08/2010	N49 17.576	W115 09.242	886	DF & PP	Unitrap	Male	Y
7942	GTAA	Elko	BC	B. Brunet	31/07/2010	21/08/2010	N49 17.576	W115 09.242	886	DF & PP	Unitrap	Male	Y
7943	ACAAA	Elko	BC	B. Brunet	31/07/2010	21/08/2010	N49 17.576	W115 09.242	886	DF & PP	Unitrap	Male	Y
7948	AGGAT	Elko	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 17.576	W115 09.242	886	DF & PP	Unitrap	Male	Y
9865	CGCTGAT	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9868	ACGACTAG	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9869	AACCGAGA	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9873	GTGAGGGT	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9874	CGAT	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9875	CTAGG	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	N
9876	GGTGT	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9877	TAATA	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9878	CCACAA	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
9879	CATCGT	Field	BC	L. Lumley	12/07/2006	24/07/2006	N51 24.38	W116 28.64	1249	ES	Unitrap	Male	Y
184	CCATGGGT	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
185	TGCA	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
187	GATG	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
188	AGCCG	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
360	GTATT	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
361	CCTAG	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
362	CCACGT	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
363	AGTGGA	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
364	GGAAGA	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	Y
376	ATTAATT	Greenwood	BC	Cdn Forest Insect & Disease Survey		15/06/1992	N49 05.4	W118 40.65	750	DF	Hand	?	N
7293	TTCTG	Hailstone Butte	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7296	ATTGA	Hailstone Butte	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7297	TCGTT	Hailstone Butte	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7298	GAGATA	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7299	CTATTA	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7300	CTTGCTT	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7801	AATATGG	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7802	GCGGAAT	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7803	TGCAAGGA	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7804	CCATGGGT	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7805	TGCA	Hailstone Butte	AB	B. Brunet	31/07/2010	21/08/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7806	GATG	Hailstone Butte	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7807	AGCCG	Hailstone Butte	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 10.461	W114 27.770	1955	WS, LP & DF	Unitrap	Male	Y
7075	CGCGATAT	Hinton	AB	B. Brunet, S. Brunet	19/06/2010	13/07/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7076	GCTGTGGA	Hinton	AB	B. Brunet, S. Brunet	19/06/2010	13/07/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7077	AACT	Hinton	AB	B. Brunet, S. Brunet	19/06/2010	13/07/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7078	CGCTT	Hinton	AB	B. Brunet, S. Brunet	19/06/2010	13/07/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7079	ACCGT	Hinton	AB	B. Brunet, S. Brunet	19/06/2010	13/07/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7081	GGAAG	Hinton	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7082	TAGGAA	Hinton	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7083	ATATGT	Hinton	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7086	GTTGAA	Hinton	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7087	GAACTTG	Hinton	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7088	CATAAGT	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	N
7089	TGCTGGA	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7090	CGCCTTAT	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7091	GGATTGGT	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7092	GCGT	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7093	TCACG	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7094	GCTTA	Hinton	AB	B. Brunet	01/08/2010	20/08/2010	N53 22.925	W 117 41.634	1046	WS	Unitrap	Male	Y
7557	ACAAA	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7559	AGGAT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7563	TACAT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7564	CTTCCA	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7565	CGCGGT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7566	TATTTTT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7568	AACGCCT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7569	CTACGGA	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7570	TAGGCCAT	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
7571	ACGTGGTA	Honeymoon Lake	AB	L. Lumley	12/07/2006	24/07/2006	N52 34.598	W117 43.453	1294	ES	Unitrap	Male	Y
9592	TCGTT	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Male	Y
9593	GAGATA	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Female	Y
9594	ACCGT	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Male	N
9595	CTATTA	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Male	Y
9596	CTTGCTT	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Male	Y
9597	AATATGG	Kalamalka Lake PP	BC	A. Roe	09/08/2005	10/08/2005	N50 12.48	W119 16.14	547		UV	Female	Y
9141	GCTCTA	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9142	ATCGTA	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9143	TAACGA	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9144	GGACCTA	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9145	CGCTGAT	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9146	ACGACTAG	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9147	AACCGAGA	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9148	GTGAGGGT	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9149	CGAT	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9150	CTAGG	Kelowna	BC	F. Sperling		14/07/2013	N49 57.66828	W119 33.58710	693	DF	Hand	Male	Y
9128	GAACTTG	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap -JPBW	Male	Y
9129	CATAAGT	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap -JPBW	Male	Y
9130	TGCTGGA	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap -JPBW	Male	Y
9131	CGCCTTAT	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9132	TGCTGGA	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9133	GGATTGGT	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9135	GCGT	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9136	TCACG	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9137	GCTTA	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
9138	GTCAA	Kettle Valley RA	BC	F. Sperling		15/07/2013	N49 06.89316	W118 58.81344	610		Unitrap	Male	Y
7253	GGTGT	Longview	AB	B. Brunet, S. Brunet	19/06/2010	12/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7254	TAATA	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7255	CCACAA	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7256	CATCGT	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7257	TGGCTA	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7258	GTCGATT	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7259	CGGTAGA	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	N
7260	TAGCATGG	Longview	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7261	ACAGGGA	Longview	AB	B. Brunet	31/07/2010	21/08/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7262	TATCGGGA	Longview	AB	B. Brunet	31/07/2010	21/08/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7264	GTAA	Longview	AB	B. Brunet	31/07/2010	21/08/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7267	ACAAA	Longview	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7271	AGGAT	Longview	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7272	TACAT	Longview	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 28.622	W114 25.407	1351	WS	Unitrap	Male	Y
7813	CATCT	Maycroft	AB	B. Brunet	12/07/2009	20/07/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7821	GGTTGT	Maycroft	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7822	ATGCCT	Maycroft	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7823	GCCAGT	Maycroft	AB	B. Brunet	17/08/2009	26/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7824	ATGAAAG	Maycroft	AB	B. Brunet	17/08/2009	26/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7826	ACGTGTT	Maycroft	AB	G. Bloom	26/08/2009	03/09/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7827	TAGCGGA	Maycroft	AB	A. Hundsdoerfer	03/09/2009	12/09/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7832	TGGTACGT	Maycroft	AB	G. Bloom	21/09/2009	30/09/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7859	CGCGATAT	Maycroft	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7860	GCTGTGGA	Maycroft	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
7872	ATATGT	Maycroft	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 50.751	W114 17.059	1451	DF	Unitrap	Male	Y
174	TGCAAGGA	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
294	ATGCCT	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
296	GCCAGT	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
297	ATGAAAG	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
298	ACGTGTT	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
6856	ACGTGTT	McBride	BC	Cdn Forest Insect & Disease Survey		04/06/1994	N53 18.0	W120 10.0	726	SF	Hand	?	Y
9812	TAGCGGA	McBride	BC	L. Lumley, J. Doucette		05/08/2008	N53 18.0	W120 10.0	726	SF	Hand	Male	Y
9813	TGGTACGT	McBride	BC	L. Lumley, J. Doucette		05/08/2008	N53 18.0	W120 10.0	726	SF	Hand	Male	Y
9814	CGCGGAGA	McBride	BC	L. Lumley, J. Doucette		05/08/2008	N53 18.0	W120 10.0	726	SF	Hand	Male	Y
9815	TCAG	McBride	BC	L. Lumley, J. Doucette		05/08/2008	N53 18.0	W120 10.0	726	SF	Hand	Male	Y
pd1.1	AACGCCT	McBride	BC	JH		26/06/1992	N53 18.0	W120 10.0	726	SF	Hand	?	Y
8001	TACAT	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8002	CTTCCA	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8003	CGCGGT	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8005	TATTTTT	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8006	AACGCCT	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8007	CTACGGA	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8010	TAGGCCAT	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8011	ACGTGGTA	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
8012	TTCCTGGA	Merritt	BC	F. Sperling		03/08/2011	N50 6.678	W120 47.173	600	DF	Hand	Female	Y
9504	AGTGGA	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9505	GGAAGA	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
9506	AAAAGTT	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9507	CGCTT	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	N
9508	ATTAATT	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9509	TCGAAGA	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9510	TCTCAGTG	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9511	CGTGTGGT	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
9512	CAGA	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	N
9513	TGCGA	Moberly Tower Rd	AB	C. Charbonneau	15/06/2012	16/10/2012	N53 32.142	W118 01.7418	1579	WS	Unitrap	Male	Y
18	CTCG	Monte Creek	BC	FIDS		20/06/1991	N50 39.0	W119 57.0	356	DF	Hand	Female	Y
89	ATTGA	Monte Creek	BC	FIDS		20/06/1991	N50 39.0	W119 57.0	356	DF	Hand	?	Y
351	CGCGGAGA	Monte Creek	BC	FIDS		20/06/1991	N50 39.0	W119 57.0	356	DF	Hand	?	Y
353	ACTA	Monte Creek	BC	FIDS		20/06/1991	N50 39.0	W119 57.0	356	DF	Hand	?	Y
354	TCAG	Monte Creek	BC	FIDS		20/06/1991	N50 39.0	W119 57.0	356	DF	Hand	?	Y
54	GTCAA	Morrisey Creek	BC	FIDS		09/07/1991	N56 19.998	W124 33	1261	SF	Hand	Female	Y
100	TCGTT	Morrisey Creek	BC	FIDS		09/07/1991	N56 19.998	W124 33	1261	SF	Hand	Male	Y
101	GAGATA	Morrisey Creek	BC	FIDS		09/07/1991	N56 19.998	W124 33	1261	SF	Hand	Male	Y
299	TAGCGGA	Morrisey Creek	BC	FIDS		09/07/1991	N56 19.998	W124 33	1261	SF	Hand	?	Y
300	TGGTACGT	Morrisey Creek	BC	FIDS		09/07/1991	N56 19.998	W124 33	1261	SF	Hand	?	Y
8976	ATCGTA	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8977	TAACGA	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8978	GGACCTA	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8981	CGCTGAT	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8983	ACGACTAG	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8984	AACCGAGA	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
8985	GTGAGGGT	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
9171	AACGCCT	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	N
9172	CTACGGA	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
9173	TAGGCCAT	Mount Shark	AB	B. McNally	07/06/2012	12/09/2012	N50 35.244	W115 21.72	2214	WS	Unitrap	Male	Y
7155	CATCT	Nordegg	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7156	GGTTGT	Nordegg	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7159	ATGCCT	Nordegg	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7160	GCCAGT	Nordegg	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7161	ATGAAAG	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	N
7162	ACGTGTT	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7164	TAGCGGA	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7166	TGGTACGT	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7167	CGCGGAGA	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	N
7168	ACTA	Nordegg	AB	B. Brunet	01/08/2010	20/08/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	N
7170	TCAG	Nordegg	AB	B. Brunet, S. Brunet	20/08/2010	04/09/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
7171	GTATT	Nordegg	AB	B. Brunet, S. Brunet	20/08/2010	04/09/2010	N52 28.700	W116 06.659	1346	WS & LP	Unitrap	Male	Y
170	CTATTA	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
171	CTTGCTT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
172	AATATGG	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
173	GCGGAAT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
291	CATCT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
292	GGTTGT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		12/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
6862	ATTAATT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		26/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	Male	Y
9930	CTTCCA	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		25/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	Male	Y
9931	CGCGGT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		25/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	Male	Y
9932	TATTTTT	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		25/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	Male	Y
pd3.1	CTACGGA	Numa Falls	BC	F. Sperling, J. Volney, J. Weber		26/06/1992	N51 07.865	W116 07.581	1411	ES & SF	Hand	?	Y
7095	GTCAA	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7096	GCTCTA	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7097	ATCGTA	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7098	TAACGA	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7099	GGACCTA	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7100	CGCTGAT	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7102	ACGACTAG	Pembina Forks	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7103	AACCGAGA	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7104	GTGAGGGT	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7105	CGAT	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7106	CTAGG	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7107	GGTGT	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7109	TAATA	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7112	CCACAA	Pembina Forks	AB	B. Brunet	01/08/2010	20/08/2010	N52 58.033	W116 36.838	1317	WS	Unitrap	Male	Y
7055	AGTGGA	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7057	GGAAGA	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7058	AAAAGTT	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7059	ATTAATT	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7060	TCGAAGA	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7061	TCTCAGTG	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7062	CGTGTGGT	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7063	CAGA	Pembina River PP	AB	B. Brunet, S. Brunet	21/06/2010	13/07/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7065	TGCGA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7066	CTGTA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7067	GAGGA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7069	TTCAGA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7070	ACCTAA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7071	GTACTT	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7072	GAATTCA	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7073	ATTGGAT	Pembina River PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7074	TCTGTGA	Pembina River PP	AB	B. Brunet	01/08/2010	20/08/2010	N53 36.357	W115 00.273	725	WS	Unitrap	Male	Y
7275	CTTCCA	Plateau Mountain	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7279	CGCGGT	Plateau Mountain	AB	B. Brunet, S. Brunet	12/07/2010	31/07/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7281	TATTTTT	Plateau Mountain	AB	B. Brunet	31/07/2010	21/08/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7283	AACGCCT	Plateau Mountain	AB	B. Brunet	31/07/2010	21/08/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7285	CTACGGA	Plateau Mountain	AB	B. Brunet	31/07/2010	21/08/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7286	TAGGCCAT	Plateau Mountain	AB	B. Brunet	31/07/2010	21/08/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7288	ACGTGGTA	Plateau Mountain	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7290	TTCCTGGA	Plateau Mountain	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7291	CTCG	Plateau Mountain	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y
7292	AGCG	Plateau Mountain	AB	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N50 14.535	W114 35.615	1757	WS & LP	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
8641	TCGAAGA	Pocaterra RA	AB	F. Sperling		10/08/2012	N50 41.322	W115 06.37242	1642	S	Hand	?	Y
8642	TCTCAGTG	Pocaterra RA	AB	F. Sperling		10/08/2012	N50 41.322	W115 06.37242	1642	S	Hand	?	Y
8644	CGTGTGGT	Pocaterra RA	AB	F. Sperling		10/08/2012	N50 41.322	W115 06.37242	1642	S	Hand	?	Y
8646	CAGA	Pocaterra RA	AB	F. Sperling		10/08/2012	N50 41.322	W115 06.37242	1642	S	Hand	?	Y
8647	TGCGA	Pocaterra RA	AB	F. Sperling		10/08/2012	N50 41.322	W115 06.37242	1642	S	Hand	?	Y
7833	CGCGGAGA	Porcupine Hills - North	AB	B. Brunet	12/07/2009	20/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7834	ACTA	Porcupine Hills - North	AB	B. Brunet	12/07/2009	20/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7835	TCAG	Porcupine Hills - North	AB	B. Brunet	12/07/2009	20/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7836	GTATT	Porcupine Hills - North	AB	B. Brunet	20/07/2009	30/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7837	CCTAG	Porcupine Hills - North	AB	B. Brunet	20/07/2009	30/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7838	CCACGT	Porcupine Hills - North	AB	B. Brunet	20/07/2009	30/07/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7839	AGTGGA	Porcupine Hills - North	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7840	GGAAGA	Porcupine Hills - North	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7841	AAAAGTT	Porcupine Hills - North	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7842	ATTAATT	Porcupine Hills - North	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7843	TCGAAGA	Porcupine Hills - North	AB	B. Brunet, B. Proshek	08/08/2009	17/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7844	TCTCAGTG	Porcupine Hills - North	AB	B. Brunet	17/08/2009	26/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7845	CGTGTGGT	Porcupine Hills - North	AB	B. Brunet	17/08/2009	26/08/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7846	CAGA	Porcupine Hills - North	AB	G. Bloom	26/08/2009	03/09/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7847	TGCGA	Porcupine Hills - North	AB	G. Bloom	26/08/2009	03/09/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7851	CTGTA	Porcupine Hills - North	AB	B. Brunet	12/09/2009	21/09/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7852	GAGGA	Porcupine Hills - North	AB	G. Bloom	21/09/2009	30/09/2009	N49 52.007	W114 00.466	1592	WS	Unitrap	Male	Y
7853	TTCAGA	Porcupine Hills - South	AB	B. Brunet	12/07/2009	20/07/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7854	ACCTAA	Porcupine Hills - South	AB	B. Brunet	12/07/2009	20/07/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7855	GTACTT	Porcupine Hills - South	AB	B. Brunet	20/07/2009	30/07/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7856	GAATTCA	Porcupine Hills - South	AB	B. Brunet	20/07/2009	30/07/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7857	ATTGGAT	Porcupine Hills - South	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7858	TCTGTGA	Porcupine Hills - South	AB	B. Brunet, S. Brunet	30/07/2009	08/08/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7862	AACT	Porcupine Hills - South	AB	B. Brunet	17/08/2009	26/08/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7864	CGCTT	Porcupine Hills - South	AB	G. Bloom	26/08/2009	03/09/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7865	ACCGT	Porcupine Hills - South	AB	A. Hundsdoerfer	03/09/2009	12/09/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7868	GGAAG	Porcupine Hills - South	AB	B. Brunet	12/09/2009	21/09/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7869	TAGGAA	Porcupine Hills - South	AB	B. Brunet	12/09/2009	21/09/2009	N49 48.573	W113 57.307	1410	DF	Unitrap	Male	Y
7173	CCTAG	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7174	CCACGT	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7176	AGTGGA	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7177	GGAAGA	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7179	AAAAGTT	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7180	ATTAATT	Ram Falls PP	AB	B. Brunet, S. Brunet	13/07/2010	01/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7181	TCGAAGA	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7182	TCTCAGTG	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7201	GCTGTGGA	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7203	AACT	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7205	CGCTT	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7206	ACCGT	Ram Falls PP	AB	B. Brunet	01/08/2010	20/08/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y
7209	GGAAG	Ram Falls PP	AB	B. Brunet, S. Brunet	20/08/2010	04/09/2010	N52 05.208	W115 52.781	1639	WS, LP & DF	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7183	CGTGTGGT	Red Lodge PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7185	CAGA	Red Lodge PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7186	TGCGA	Red Lodge PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7187	CTGTA	Red Lodge PP	AB	B. Brunet, S. Brunet	14/07/2010	01/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7189	GAGGA	Red Lodge PP	AB	B. Brunet	01/08/2010	21/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7192	TTCAGA	Red Lodge PP	AB	B. Brunet	01/08/2010	21/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7193	ACCTAA	Red Lodge PP	AB	B. Brunet	01/08/2010	21/08/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7211	TAGGAA	Red Lodge PP	AB	B. Brunet	20/06/2010	14/07/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7212	ATATGT	Red Lodge PP	AB	B. Brunet	20/06/2010	14/07/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7213	GTTGAA	Red Lodge PP	AB	B. Brunet	20/06/2010	14/07/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7214	GAACTTG	Red Lodge PP	AB	B. Brunet	20/06/2010	14/07/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
7215	CATAAGT	Red Lodge PP	AB	B. Brunet	20/06/2010	14/07/2010	N51 56.683	W114 14.290	954	WS	Unitrap	Male	Y
9855	TGCTGGA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9856	CGCCTTAT	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9857	GGATTGGT	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9858	TCACG	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9859	GCTTA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9860	GTCAA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9861	GCTCTA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9862	ATCGTA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9863	TAACGA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
9864	GGACCTA	Saskatchewan River Crossing	AB	L. Lumley		12/07/2006	N51 58.378	W116 44.097	1457	ES & LP	Unitrap -JPBW	Male	Y
8966	CGCCTTAT	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8967	GGATTGGT	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8968	ATATGT	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8969	GTTGAA	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8970	GAACTTG	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8971	GCGT	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8972	TCACG	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8973	GCTTA	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8974	GTCAA	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
8975	GCTCTA	Sibbald	AB	B. McNally	07/06/2012	20/09/2012	N51 03.13920	W114 56.547	1554	WS	Unitrap	Male	Y
9598	GCGGAAT	Smith Lake	AB	L. Lumley		12/07/2006	N51 15.658	W115 56.009	1441	ES & LP	Unitrap -JPBW	Male	Y
9599	TGCAAGGA	Smith Lake	AB	L. Lumley		12/07/2006	N51 15.658	W115 56.009	1441	ES & LP	Unitrap -JPBW	Male	Y
9600	CCATGGGT	Smith Lake	AB	L. Lumley		12/07/2006	N51 15.658	W115 56.009	1441	ES & LP	Unitrap -JPBW	Male	Y
9801	GATG	Smith Lake	AB	L. Lumley		12/07/2006	N51 15.658	W115 56.009	1441	ES & LP	Unitrap -JPBW	Male	Y
9802	AGCCG	Smith Lake	AB	L. Lumley		12/07/2006	N51 15.658	W115 56.009	1441	ES & LP	Unitrap -JPBW	Male	Y
7916	CGAT	Sparwood	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7917	CTAGG	Sparwood	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7919	GGTGT	Sparwood	BC	B. Brunet, S. Brunet	11/07/2010	31/07/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7920	TAATA	Sparwood	BC	B. Brunet	31/07/2010	21/08/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7926	CCACAA	Sparwood	BC	B. Brunet	31/07/2010	21/08/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7927	CATCGT	Sparwood	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7928	TGGCTA	Sparwood	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7929	GTCGATT	Sparwood	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7930	CGGTAGA	Sparwood	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	N

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-1: (continued)

ID	Barcode	Location	Prov	Coll	Dep. Date	Coll. Date	Latitude	Longitude	Elev	Host	Method	Sex	Filt
7932	TAGCATGG	Sparwood	BC	B. Brunet, S. Brunet	21/08/2010	05/09/2010	N49 43.557	W114 51.100	1248	WS	Unitrap	Male	Y
7195	GTACTT	Strawberry Ridge	AB	B. Brunet, S. Brunet	12/07/2010	01/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7196	GAATTCA	Strawberry Ridge	AB	B. Brunet, S. Brunet	12/07/2010	01/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7198	ATTGGAT	Strawberry Ridge	AB	B. Brunet, S. Brunet	12/07/2010	01/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7199	TCTGTGA	Strawberry Ridge	AB	B. Brunet, S. Brunet	12/07/2010	01/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7200	CGCGATAT	Strawberry Ridge	AB	B. Brunet, S. Brunet	12/07/2010	01/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7220	TGCTGGA	Strawberry Ridge	AB	B. Brunet	01/08/2010	20/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7222	CGCCTTAT	Strawberry Ridge	AB	B. Brunet	01/08/2010	20/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7223	GGATTGGT	Strawberry Ridge	AB	B. Brunet	01/08/2010	20/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7224	GCGT	Strawberry Ridge	AB	B. Brunet	01/08/2010	20/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
7225	TCACG	Strawberry Ridge	AB	B. Brunet	01/08/2010	20/08/2010	N51 46.566	W115 14.886	1426	WS & LP	Unitrap	Male	Y
8913	GAGGA	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y
8918	TTCAGA	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y
9116	CGCGATAT	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	N
9117	GCTGTGGA	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	N
9118	AACT	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y
9119	CGCTT	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	N
9121	ACCGT	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y
9122	GGAAG	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y
9124	CATAAGT	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	N
9125	TAGGAA	Wolf Lake Road	AB	C. Charbonneau	15/06/2012	21/09/2012	N53 29.48760	W116 04.05360	962	WS	Unitrap	Male	Y

ID is specimen DNA#; Barcode is that used in GBS library; Location of collection; Province of collection; Collector; Trap deployment date; Trap collection date; Latitude, longitude and elevation of collection location; Host species collected from or near (WS=white spruce, ES=engelmann spruce; BS=blue spruce; S=spruce; JP=jack pine; LP=lodgepole pine; PP=ponderosa pine; LA=larch; SF=subalpine fir; DF=Douglas fir); Collection method (UV light trap or Pheromone unitrap with either jackpine budworm lure or spruce budworm lure); sex; Filt = (Y/N) sample pass GBS quality filters

Appendix 4-2: Symmetrical matrix of pairwise population genetic differentiation (Fst) (below diagonal) estimates and corresponding p-values (above diagonal) deduced by 999 permutations in an AMOVA framework. Population names are the same as in Table 4-1.

	BD	BM	BC	BL	BR	CF	Cs	Cm	CL	EP	Ew	Ed	Ek	Fd	Gw	HB	Ht	HL	KL	Kw	KV	Lv	Mc
BD	0.00	0.02	0.01	0.00	0.01	0.23	0.10	0.03	0.00	0.00	0.24	0.00	0.06	0.16	0.11	0.03	0.03	0.23	0.10	0.12	0.10	0.00	0.02
BM	0.04	0.00	0.02	0.00	0.35	0.03	0.10	0.35	0.00	0.00	0.05	0.00	0.32	0.09	0.10	0.19	0.07	0.03	0.21	0.06	0.06	0.00	0.35
BC	0.06	0.04	0.00	0.00	0.01	0.01	0.01	0.02	0.12	0.00	0.00	0.00	0.10	0.01	0.01	0.29	0.01	0.00	0.06	0.01	0.00	0.38	0.00
BL	0.37	0.38	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BR	0.04	0.00	0.05	0.39	0.00	0.01	0.07	0.36	0.00	0.00	0.04	0.00	0.32	0.03	0.08	0.08	0.06	0.01	0.20	0.05	0.04	0.00	0.32
CF	0.01	0.04	0.06	0.38	0.04	0.00	0.09	0.02	0.00	0.00	0.29	0.00	0.06	0.12	0.14	0.03	0.03	0.35	0.08	0.12	0.12	0.00	0.02
Cs	0.02	0.02	0.06	0.38	0.02	0.02	0.00	0.09	0.00	0.00	0.21	0.00	0.15	0.09	0.19	0.03	0.02	0.10	0.25	0.13	0.17	0.00	0.10
Cm	0.04	0.00	0.04	0.39	0.00	0.04	0.02	0.00	0.00	0.00	0.08	0.00	0.30	0.08	0.08	0.20	0.08	0.02	0.20	0.07	0.09	0.00	0.33
CL	0.15	0.14	0.02	0.08	0.15	0.16	0.15	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00
EP	0.35	0.34	0.17	0.01	0.35	0.36	0.35	0.35	0.06	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ew	0.01	0.03	0.06	0.38	0.03	0.00	0.01	0.03	0.16	0.35	0.00	0.00	0.06	0.12	0.20	0.03	0.02	0.28	0.14	0.22	0.21	0.00	0.03
Ed	0.39	0.39	0.21	0.01	0.40	0.41	0.40	0.40	0.09	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ek	0.04	0.00	0.04	0.39	0.00	0.04	0.02	0.00	0.14	0.35	0.03	0.40	0.00	0.08	0.16	0.32	0.09	0.06	0.20	0.06	0.08	0.01	0.30
Fd	0.01	0.02	0.05	0.38	0.03	0.01	0.02	0.03	0.15	0.35	0.01	0.40	0.03	0.00	0.09	0.08	0.13	0.12	0.21	0.09	0.08	0.00	0.06
Gw	0.02	0.02	0.06	0.39	0.02	0.01	0.01	0.02	0.16	0.36	0.01	0.41	0.02	0.02	0.00	0.05	0.02	0.11	0.24	0.23	0.30	0.00	0.11
HB	0.03	0.01	0.00	0.29	0.02	0.03	0.03	0.01	0.08	0.26	0.03	0.31	0.00	0.02	0.03	0.00	0.11	0.02	0.15	0.01	0.01	0.01	0.09
Ht	0.03	0.02	0.04	0.38	0.02	0.03	0.04	0.02	0.15	0.35	0.03	0.39	0.02	0.01	0.04	0.01	0.00	0.04	0.08	0.01	0.01	0.00	0.07
HL	0.01	0.04	0.06	0.38	0.04	0.00	0.02	0.04	0.16	0.36	0.00	0.40	0.05	0.01	0.02	0.03	0.03	0.00	0.06	0.12	0.10	0.00	0.02
KL	0.03	0.01	0.04	0.39	0.01	0.03	0.01	0.01	0.14	0.35	0.02	0.41	0.01	0.01	0.01	0.02	0.03	0.03	0.00	0.20	0.20	0.01	0.18
Kw	0.02	0.03	0.07	0.39	0.03	0.02	0.01	0.03	0.17	0.36	0.01	0.41	0.03	0.02	0.01	0.04	0.04	0.02	0.02	0.00	0.35	0.00	0.04
KV	0.02	0.03	0.07	0.39	0.03	0.02	0.01	0.03	0.17	0.36	0.01	0.41	0.03	0.02	0.00	0.04	0.04	0.02	0.01	0.00	0.00	0.00	0.04
Lv	0.12	0.11	0.00	0.11	0.12	0.13	0.12	0.11	0.00	0.08	0.13	0.12	0.10	0.11	0.13	0.05	0.11	0.13	0.11	0.14	0.13	0.00	0.00
Mc	0.04	0.00	0.05	0.39	0.00	0.04	0.02	0.00	0.15	0.35	0.03	0.40	0.00	0.02	0.02	0.01	0.02	0.05	0.02	0.03	0.03	0.12	0.00
Mb	0.00	0.03	0.05	0.38	0.03	0.01	0.02	0.03	0.15	0.35	0.01	0.39	0.03	0.00	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.12	0.03
Mt	0.04	0.00	0.04	0.38	0.00	0.04	0.02	0.00	0.14	0.35	0.03	0.39	0.00	0.02	0.02	0.01	0.02	0.04	0.00	0.03	0.03	0.11	0.01
My	0.01	0.04	0.05	0.37	0.04	0.01	0.02	0.04	0.14	0.34	0.01	0.39	0.04	0.01	0.03	0.02	0.03	0.01	0.03	0.03	0.03	0.11	0.04
Mn	0.03	0.02	0.05	0.39	0.02	0.02	0.01	0.02	0.14	0.36	0.01	0.41	0.02	0.02	0.00	0.02	0.03	0.03	0.00	0.01	0.01	0.11	0.02
Mr	0.00	0.04	0.05	0.38	0.04	0.01	0.02	0.04	0.14	0.35	0.00	0.40	0.04	0.01	0.02	0.02	0.02	0.00	0.03	0.02	0.02	0.11	0.04
MS	0.00	0.04	0.05	0.37	0.04	0.00	0.02	0.04	0.14	0.34	0.00	0.39	0.04	0.01	0.02	0.02	0.03	0.00	0.03	0.02	0.02	0.11	0.04
Nd	0.03	0.02	0.00	0.25	0.03	0.04	0.04	0.03	0.04	0.22	0.04	0.27	0.02	0.03	0.04	0.00	0.02	0.04	0.02	0.05	0.05	0.02	0.03
NF	0.00	0.03	0.05	0.38	0.03	0.01	0.02	0.03	0.15	0.35	0.01	0.40	0.03	0.00	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.12	0.03
PF	0.03	0.02	0.00	0.29	0.02	0.03	0.04	0.02	0.08	0.26	0.03	0.31	0.01	0.02	0.04	0.00	0.01	0.03	0.02	0.04	0.04	0.05	0.02
PR	0.35	0.35	0.18	0.01	0.36	0.36	0.36	0.36	0.06	0.00	0.36	0.00	0.36	0.36	0.37	0.27	0.35	0.36	0.36	0.37	0.37	0.09	0.36
PM	0.03	0.01	0.04	0.39	0.01	0.03	0.03	0.01	0.14	0.35	0.03	0.40	0.01	0.01	0.03	0.01	0.00	0.03	0.02	0.04	0.04	0.11	0.02
Pc	0.01	0.04	0.04	0.37	0.04	0.01	0.02	0.04	0.14	0.35	0.01	0.40	0.04	0.01	0.02	0.02	0.03	0.01	0.02	0.03	0.02	0.11	0.04
P1	0.04	0.00	0.05	0.39	0.00	0.04	0.02	0.00	0.16	0.35	0.03	0.40	0.00	0.02	0.02	0.01	0.02	0.04	0.02	0.03	0.03	0.12	0.00
P2	0.04	0.00	0.05	0.39	0.00	0.04	0.03	0.00	0.15	0.35	0.03	0.40	0.01	0.03	0.02	0.01	0.02	0.05	0.02	0.03	0.03	0.12	0.00
RF	0.02	0.01	0.04	0.38	0.01	0.03	0.03	0.02	0.14	0.34	0.03	0.39	0.02	0.01	0.03	0.01	0.00	0.03	0.02	0.04	0.04	0.11	0.02
RL	0.27	0.26	0.10	0.02	0.28	0.28	0.27	0.27	0.01	0.00	0.28	0.02	0.27	0.27	0.28	0.19	0.27	0.28	0.27	0.29	0.29	0.03	0.28
SX	0.06	0.07	0.08	0.39	0.06	0.06	0.05	0.07	0.17	0.35	0.05	0.40	0.07	0.05	0.06	0.06	0.07	0.06	0.05	0.07	0.06	0.13	0.07
Sb	0.00	0.04	0.06	0.38	0.03	0.00	0.02	0.04	0.15	0.35	0.00	0.40	0.04	0.01	0.01	0.03	0.02	0.00	0.02	0.01	0.02	0.12	0.04
SL	0.05	0.06	0.05	0.39	0.06	0.05	0.05	0.06	0.14	0.35	0.05	0.40	0.07	0.04	0.05	0.04	0.05	0.06	0.05	0.06	0.06	0.11	0.06
Sw	0.04	0.00	0.05	0.39	0.00	0.04	0.02	0.00	0.15	0.35	0.03	0.40	0.00	0.03	0.02	0.01	0.02	0.05	0.01	0.03	0.03	0.12	0.00
SR	0.03	0.01	0.02	0.35	0.01	0.03	0.03	0.02	0.12	0.32	0.03	0.37	0.01	0.01	0.03	0.00	0.00	0.03	0.02	0.04	0.03	0.08	0.01
WL	0.02	0.04	0.01	0.27	0.05	0.02	0.03	0.04	0.06	0.25	0.02	0.30	0.04	0.02	0.03	0.01	0.04	0.02	0.03	0.03	0.03	0.04	0.05

Appendix 4-2: (continued)

	Mb	Mt	My	Mn	Mr	MS	Nd	NF	PF	PR	PM	Pc	P1	P2	RF	RL	SX	Sb	SL	Sw	SR	WL
BD	0.24	0.03	0.18	0.10	0.29	0.33	0.03	0.27	0.04	0.00	0.07	0.22	0.01	0.03	0.05	0.00	0.01	0.31	0.05	0.03	0.06	0.17
BM	0.04	0.29	0.02	0.16	0.06	0.03	0.07	0.04	0.06	0.00	0.13	0.05	0.35	0.34	0.14	0.00	0.00	0.03	0.03	0.28	0.12	0.06
BC	0.00	0.02	0.01	0.05	0.05	0.01	0.37	0.01	0.38	0.00	0.02	0.07	0.00	0.00	0.02	0.00	0.00	0.00	0.04	0.01	0.10	0.30
BL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
BR	0.02	0.23	0.03	0.12	0.05	0.02	0.03	0.04	0.03	0.00	0.15	0.04	0.33	0.25	0.08	0.00	0.00	0.02	0.01	0.28	0.14	0.02
CF	0.21	0.03	0.15	0.11	0.25	0.30	0.02	0.21	0.01	0.00	0.06	0.25	0.01	0.02	0.04	0.00	0.01	0.35	0.05	0.02	0.04	0.11
Cs	0.08	0.11	0.09	0.25	0.17	0.09	0.03	0.11	0.01	0.00	0.04	0.12	0.06	0.09	0.02	0.00	0.01	0.11	0.05	0.07	0.06	0.10
Cm	0.05	0.28	0.05	0.18	0.04	0.06	0.08	0.07	0.06	0.00	0.10	0.04	0.34	0.32	0.13	0.00	0.01	0.03	0.04	0.27	0.10	0.04
CL	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.02
EP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00
Ew	0.18	0.06	0.12	0.20	0.28	0.31	0.02	0.19	0.03	0.00	0.04	0.24	0.04	0.04	0.04	0.00	0.01	0.32	0.04	0.05	0.04	0.13
Ed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Ek	0.06	0.32	0.06	0.16	0.10	0.05	0.15	0.06	0.17	0.00	0.19	0.07	0.29	0.26	0.11	0.00	0.01	0.05	0.02	0.30	0.20	0.07
Fd	0.28	0.09	0.20	0.18	0.24	0.16	0.04	0.25	0.10	0.00	0.12	0.18	0.06	0.04	0.12	0.00	0.02	0.17	0.05	0.10	0.10	0.09
Gw	0.11	0.10	0.09	0.31	0.21	0.11	0.02	0.11	0.02	0.00	0.05	0.14	0.07	0.08	0.03	0.00	0.01	0.12	0.05	0.10	0.05	0.06
HB	0.05	0.15	0.06	0.12	0.10	0.06	0.37	0.05	0.37	0.00	0.23	0.10	0.07	0.14	0.19	0.00	0.00	0.04	0.05	0.13	0.41	0.23
Ht	0.08	0.07	0.04	0.07	0.08	0.04	0.06	0.06	0.15	0.00	0.23	0.05	0.06	0.05	0.22	0.00	0.00	0.05	0.02	0.04	0.26	0.03
HL	0.17	0.03	0.15	0.09	0.31	0.26	0.02	0.20	0.02	0.00	0.04	0.23	0.01	0.03	0.04	0.00	0.01	0.25	0.04	0.02	0.03	0.10
KL	0.15	0.30	0.13	0.28	0.17	0.10	0.11	0.22	0.11	0.00	0.11	0.17	0.11	0.19	0.12	0.00	0.04	0.13	0.06	0.20	0.15	0.12
Kw	0.08	0.07	0.06	0.25	0.17	0.10	0.01	0.11	0.01	0.00	0.01	0.14	0.03	0.04	0.01	0.00	0.00	0.13	0.04	0.06	0.02	0.07
KV	0.07	0.06	0.07	0.21	0.16	0.12	0.01	0.10	0.00	0.00	0.03	0.13	0.05	0.05	0.01	0.00	0.00	0.12	0.04	0.07	0.03	0.09
Lv	0.00	0.00	0.00	0.00	0.01	0.00	0.10	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.05
Mc	0.03	0.22	0.02	0.10	0.07	0.02	0.03	0.04	0.04	0.00	0.10	0.05	0.31	0.30	0.10	0.00	0.01	0.04	0.02	0.28	0.13	0.03
Mb	0.00	0.04	0.19	0.20	0.33	0.31	0.04	0.33	0.06	0.00	0.11	0.24	0.01	0.04	0.07	0.00	0.01	0.30	0.05	0.03	0.12	0.12
Mt	0.03	0.00	0.03	0.22	0.05	0.03	0.05	0.05	0.07	0.00	0.14	0.08	0.21	0.20	0.12	0.00	0.01	0.03	0.05	0.21	0.10	0.06
My	0.01	0.04	0.00	0.09	0.23	0.20	0.07	0.17	0.06	0.00	0.10	0.20	0.04	0.02	0.06	0.00	0.01	0.17	0.05	0.02	0.09	0.17
Mn	0.01	0.01	0.03	0.00	0.16	0.09	0.07	0.19	0.07	0.00	0.07	0.19	0.09	0.08	0.06	0.00	0.03	0.10	0.04	0.11	0.07	0.15
Mr	0.00	0.03	0.01	0.02	0.00	0.28	0.12	0.30	0.13	0.00	0.07	0.28	0.07	0.06	0.06	0.00	0.03	0.28	0.04	0.05	0.10	0.22
MS	0.00	0.04	0.01	0.03	0.00	0.00	0.04	0.24	0.06	0.00	0.07	0.26	0.02	0.02	0.07	0.00	0.01	0.30	0.04	0.03	0.08	0.20
Nd	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.04	0.39	0.00	0.11	0.13	0.03	0.04	0.09	0.00	0.00	0.02	0.05	0.04	0.28	0.37
NF	0.00	0.02	0.01	0.01	0.00	0.00	0.03	0.00	0.04	0.00	0.10	0.28	0.04	0.04	0.12	0.00	0.01	0.27	0.04	0.03	0.11	0.11
PF	0.02	0.02	0.02	0.03	0.02	0.02	0.00	0.02	0.00	0.00	0.22	0.11	0.02	0.05	0.20	0.00	0.00	0.04	0.05	0.06	0.38	0.21
PR	0.35	0.35	0.35	0.36	0.36	0.35	0.23	0.36	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00
PM	0.01	0.01	0.02	0.03	0.03	0.02	0.02	0.01	0.01	0.35	0.00	0.07	0.13	0.10	0.34	0.00	0.00	0.09	0.04	0.12	0.36	0.05
Pc	0.01	0.03	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.35	0.02	0.00	0.04	0.04	0.07	0.00	0.03	0.24	0.06	0.06	0.06	0.24
P1	0.03	0.00	0.04	0.02	0.04	0.04	0.03	0.02	0.02	0.36	0.01	0.04	0.00	0.24	0.07	0.00	0.00	0.02	0.01	0.28	0.11	0.02
P2	0.03	0.01	0.04	0.03	0.04	0.04	0.03	0.03	0.02	0.35	0.02	0.04	0.00	0.00	0.11	0.00	0.00	0.03	0.03	0.24	0.12	0.03
RF	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.01	0.35	0.00	0.03	0.01	0.02	0.00	0.00	0.00	0.05	0.03	0.12	0.29	0.06
RL	0.27	0.27	0.26	0.27	0.27	0.26	0.14	0.27	0.19	0.00	0.27	0.26	0.28	0.27	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SX	0.05	0.06	0.06	0.05	0.06	0.06	0.06	0.05	0.06	0.36	0.06	0.06	0.06	0.07	0.06	0.28	0.00	0.01	0.22	0.01	0.01	0.01
Sb	0.00	0.03	0.01	0.03	0.00	0.00	0.04	0.00	0.03	0.36	0.02	0.01	0.03	0.04	0.02	0.28	0.06	0.00	0.05	0.02	0.10	0.11
SL	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.36	0.05	0.05	0.06	0.06	0.05	0.26	0.01	0.05	0.00	0.04	0.04	0.08
Sw	0.03	0.01	0.04	0.02	0.04	0.04	0.03	0.03	0.02	0.36	0.01	0.04	0.00	0.00	0.02	0.28	0.06	0.03	0.06	0.00	0.10	0.04
SR	0.01	0.01	0.02	0.03	0.02	0.02	0.00	0.02	0.00	0.32	0.00	0.03	0.01	0.02	0.00	0.24	0.06	0.02	0.05	0.02	0.00	0.08
WL	0.02	0.04	0.02	0.02	0.01	0.01	0.00	0.02	0.01	0.26	0.04	0.01	0.05	0.05	0.03	0.17	0.06	0.02	0.04	0.05	0.02	0.00

Appendix 4-3: Locus-specific observed (Ho) and unbiased expected heterozygosity (uHe), and fixation index (F), as well as the frequency of allele A1 in each species deduced from reference population frequencies.

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S1_781981	T	C	0.04	0.09	0.43	1.00	0.00	0.00
S10_103916	T	C	0.19	0.19	-0.05	0.00	0.10	0.09
S10002_7403	T	C	0.12	0.10	-0.20	0.02	0.00	0.22
S10002_7440	A	G	0.49	0.49	-0.07	0.50	0.60	0.66
S10006_6853	A	G	0.14	0.14	-0.03	0.00	0.12	0.03
S10072_3672	A	C	0.19	0.26	0.15	0.00	0.92	0.84
S10081_4687	A	G	0.43	0.39	-0.13	0.27	0.35	0.34
S10090_6314	T	C	0.27	0.31	0.09	0.00	0.13	0.38
S10090_6317	G	C	0.15	0.16	0.01	0.00	0.19	0.03
S1010_30342	T	G	0.14	0.15	-0.01	0.34	0.10	0.00
S1010_30348	A	G	0.25	0.24	-0.08	0.00	0.13	0.16
S1014_21807	T	G	0.06	0.08	0.10	0.36	0.00	0.03
S10145_6688	T	C	0.28	0.29	-0.05	0.27	0.10	0.16
S1015_31683	T	C	0.15	0.15	-0.07	0.00	0.10	0.09
S1015_31718	T	G	0.19	0.16	-0.19	1.00	0.79	0.88
S1015_31777	T	C	0.25	0.23	-0.10	0.98	0.87	0.91
S10186_7175	A	G	0.25	0.31	0.12	0.12	0.21	0.13
S10186_7193	A	G	0.12	0.17	0.15	0.05	0.13	0.03
S10186_7203	T	C	0.09	0.15	0.26	0.07	0.12	0.09
S10205_5665	A	T	0.10	0.10	-0.09	0.91	0.94	0.97
S10205_5681	T	G	0.50	0.36	-0.41	0.75	0.65	0.84
S10217_1616	T	G	0.09	0.09	-0.01	0.00	0.02	0.13
S10224_5277	A	T	0.26	0.30	0.07	0.05	0.25	0.28
S10249_5829	A	T	0.16	0.15	-0.11	0.11	0.06	0.13
S10249_5835	A	C	0.15	0.14	-0.13	0.00	0.04	0.06
S10249_5837	T	C	0.12	0.11	-0.10	0.09	0.02	0.09
S10249_5844	A	T	0.84	0.51	-0.75	0.64	0.58	0.56
S10249_5847	A	C	0.79	0.49	-0.68	0.70	0.54	0.53
S1025_10643	T	C	0.13	0.16	0.10	0.00	0.08	0.03
S1025_10694	A	T	0.13	0.15	0.02	0.00	0.12	0.06
S1025_10715	T	C	0.13	0.13	-0.03	0.05	0.06	0.16
S1025_10729	A	G	0.10	0.12	0.10	0.55	0.00	0.00
S10254_1156	A	G	0.07	0.07	0.05	0.73	0.98	1.00
S10254_1312	T	C	0.18	0.22	0.14	0.14	0.17	0.28
S10254_1340	G	C	0.13	0.14	-0.01	0.00	0.12	0.03
S1028_38265	T	G	0.02	0.09	0.54	0.98	0.02	0.00
S10292_5767	A	G	0.08	0.09	0.01	0.23	0.02	0.03
S10318_6105	A	G	0.08	0.09	-0.01	0.23	0.00	0.06
S10318_6125	A	C	0.20	0.19	-0.06	1.00	0.88	0.84
S10318_6128	A	T	0.17	0.19	0.03	0.02	0.15	0.13
S10318_6144	T	C	0.20	0.21	-0.04	0.07	0.13	0.16
S10318_6145	T	C	0.09	0.09	-0.02	0.32	0.02	0.00
S10359_149	T	C	0.13	0.13	-0.03	0.20	0.04	0.09
S1038_8778	A	G	0.12	0.12	-0.03	0.95	0.96	0.81
S1038_8805	A	T	0.17	0.18	0.00	0.34	0.96	1.00
S1038_8810	A	T	0.17	0.18	-0.02	0.86	0.96	0.94
S1039_11506	A	G	0.09	0.10	-0.01	0.41	0.00	0.03
S1039_11518	A	C	0.10	0.10	-0.06	0.00	0.04	0.03
S1040_38991	A	G	0.21	0.20	-0.06	0.05	0.12	0.09
S1040_38995	A	T	0.19	0.21	-0.01	0.00	0.10	0.22
S1042_25402	A	G	0.08	0.12	0.29	0.50	1.00	0.94
S10430_5250	A	C	0.10	0.14	0.12	1.00	0.96	0.78
S10472_3196	T	C	0.39	0.31	-0.27	0.70	0.73	0.72
S10486_297	A	T	0.17	0.20	0.03	0.34	0.96	0.94
S1049_41070	T	C	0.16	0.17	-0.01	1.00	0.85	0.81
S1049_41101	T	C	0.17	0.17	-0.03	0.00	0.06	0.09
S1050_29030	A	C	0.14	0.13	-0.16	0.91	0.79	0.97
S1050_29031	A	T	0.10	0.10	-0.04	0.00	0.04	0.13
S1050_29034	A	G	0.15	0.15	-0.06	0.16	0.12	0.09
S1057_28284	A	G	0.16	0.14	-0.18	0.84	0.69	0.97
S1057_28288	T	G	0.58	0.35	-0.60	0.89	0.77	0.50
S1059_12285	T	G	0.16	0.14	-0.14	0.05	0.06	0.00
S1059_12288	T	C	0.34	0.28	-0.24	0.84	0.83	0.81
S1059_12306	T	C	0.13	0.12	-0.10	0.00	0.17	0.03
S1059_12314	G	C	0.18	0.16	-0.15	0.91	0.87	0.97
S1059_12325	T	G	0.30	0.26	-0.19	0.00	0.15	0.22
S1060_7583	T	C	0.12	0.18	0.20	0.02	0.83	0.94
S10620_6680	A	T	0.16	0.15	-0.09	0.86	0.94	0.94
S1063_15533	A	T	0.16	0.13	-0.22	0.02	0.21	0.00
S1063_15830	T	C	0.10	0.12	0.09	0.43	0.06	0.03
S1063_15869	A	C	0.14	0.15	-0.02	0.00	0.08	0.16
S1063_18234	T	G	0.21	0.16	-0.27	0.95	0.96	0.84
S10658_306	A	G	0.17	0.18	-0.04	0.02	0.13	0.16
S1069_27444	T	C	0.10	0.12	0.06	0.00	0.13	0.13
S1073_12583	T	G	0.32	0.30	-0.12	0.86	0.83	0.72
S1073_27381	T	C	0.09	0.11	0.03	0.39	0.00	0.00
S1079_27608	A	C	0.04	0.09	0.33	0.02	0.98	1.00
S10790_6252	T	G	0.15	0.14	-0.06	0.05	0.08	0.09
S10790_6257	A	G	0.10	0.10	-0.08	0.91	0.96	0.97
S108_21724	G	C	0.12	0.12	-0.01	0.02	0.04	0.03
S108_21740	G	C	0.15	0.15	-0.05	1.00	0.92	0.81
S1081_41816	T	G	0.09	0.13	0.23	0.50	1.00	0.97
S1082_16067	A	T	0.14	0.11	-0.20	0.93	0.77	1.00
S1083_32522	T	C	0.01	0.07	0.66	1.00	0.00	0.00
S1083_32535	A	C	0.12	0.13	0.06	0.00	0.06	0.00
S1083_32548	A	G	0.11	0.13	0.02	1.00	0.94	0.84
S1084_3270	T	C	0.39	0.42	0.03	0.80	0.67	0.66
S1088_23133	T	C	0.27	0.28	-0.02	0.02	0.27	0.22
S1089_5315	A	T	0.24	0.22	-0.13	0.80	0.83	0.88
S1089_5318	T	C	0.72	0.47	-0.56	0.16	0.54	0.53
S1089_5325	A	G	0.30	0.25	-0.20	0.93	0.85	0.78
S1089_5333	T	C	0.48	0.37	-0.34	0.27	0.15	0.38
S1089_5377	A	G	0.41	0.26	-0.62	0.80	0.58	0.97
S109_16914	T	C	0.14	0.14	-0.04	0.05	0.02	0.06
S10906_5100	A	T	0.13	0.15	0.08	0.02	0.19	0.03
S1093_15778	A	C	0.03	0.10	0.40	1.00	0.00	0.00
S1093_15802	A	T	0.12	0.11	-0.10	1.00	0.88	0.97
S10933_2205	T	C	0.16	0.18	0.05	0.00	0.10	0.13
S10939_6182	A	G	0.42	0.45	0.01	0.07	0.44	0.50
S10939_7323	T	G	0.24	0.25	-0.03	0.07	0.19	0.13
S10963_7445	A	G	0.29	0.29	-0.07	0.89	0.85	0.88
S10963_7446	T	G	0.10	0.12	0.05	0.00	0.12	0.03
S1097_28483	A	G	0.39	0.41	0.01	0.93	0.60	0.47
S11_34313	T	C	0.28	0.30	-0.05	0.30	0.87	0.84
S110_114424	T	G	0.14	0.12	-0.15	0.95	0.88	1.00
S110_114440	T	C	0.10	0.10	-0.05	0.05	0.06	0.03
S110_114443	T	C	0.12	0.12	-0.04	0.05	0.02	0.06
S110_20153	T	G	0.14	0.17	0.08	0.86	0.83	0.97
S1101_1761	A	C	0.29	0.29	-0.07	1.00	0.75	0.78
S1103_19660	A	G	0.05	0.11	0.28	0.02	1.00	0.97
S1103_19705	A	G	0.14	0.14	-0.07	0.00	0.06	0.03
S11071_5544	G	C	0.10	0.11	-0.02	1.00	0.96	0.97
S11071_5568	T	C	0.19	0.24	0.10	0.93	0.81	0.69
S11083_2551	T	C	0.02	0.08	0.61	0.98	0.00	0.00
S11091_1006	A	G	0.10	0.10	-0.03	0.30	0.00	0.06
S11091_1043	A	C	0.15	0.14	-0.09	0.05	0.13	0.16
S1121_5077	T	C	0.11	0.12	0.05	0.00	0.10	0.03
S11226_353	T	C	0.13	0.14	0.01	0.00	0.08	0.13
S1128_942	T	C	0.26	0.35	0.19	0.00	0.31	0.09
S1133_728	A	G	0.08	0.11	0.13	0.02	0.06	0.09
S1133_738	A	G	0.28	0.37	0.17	0.02	0.74	0.78
S11335_3334	A	G	0.19	0.18	-0.06	0.00	0.19	0.09
S11361_6791	G	C	0.46	0.47	-0.02	0.52	0.50	0.56
S11361_6815	T	C	0.12	0.14	0.02	0.00	0.12	0.06
S1138_10374	T	C	0.15	0.22	0.23	0.12	0.90	1.00
S1138_18740	T	C	0.14	0.15	-0.01	0.89	0.92	0.91
S1139_24849	T	C	0.11	0.13	0.01	0.00	0.10	0.19
S11416_6035	A	T	0.07	0.09	0.06	0.95	0.96	0.94
S11416_6093	T	C	0.11	0.12	-0.03	0.89	0.96	0.97
S1145_793	T	C	0.18	0.19	-0.02	0.11	0.21	0.13

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S11464_540	G	C	0.07	0.07	0.03	0.27	0.00	0.00
S11517_5449	A	G	0.12	0.13	0.02	0.02	0.15	0.06
S11530_3435	T	C	0.41	0.43	-0.01	0.50	0.60	0.53
S11538_3505	T	C	0.07	0.11	0.22	0.80	0.00	0.03
S1154_18228	A	G	0.08	0.09	0.06	0.59	0.98	1.00
S1154_18252	T	G	0.08	0.08	-0.03	0.68	1.00	0.94
S1154_24799	T	C	0.24	0.23	-0.08	0.98	0.69	0.84
S1156_10278	A	C	0.18	0.14	-0.28	0.05	0.00	0.31
S1156_10292	T	C	0.36	0.44	0.13	0.05	0.68	0.63
S1156_10309	T	G	0.10	0.11	0.05	0.00	0.10	0.00
S1158_20839	A	G	0.34	0.33	-0.08	0.91	0.71	0.88
S11588_1172	A	G	0.40	0.48	0.11	0.61	0.31	0.41
S11588_1201	T	C	0.06	0.08	0.10	0.20	0.04	0.03
S11589_2189	T	G	0.33	0.23	-0.41	0.95	1.00	0.59
S11599_5613	A	C	0.39	0.44	0.07	0.00	0.46	0.56
S116_38372	A	T	0.08	0.12	0.22	0.07	0.96	0.97
S11609_2551	T	C	0.07	0.10	0.29	0.50	0.00	0.00
S11686_2436	A	G	0.01	0.07	0.78	1.00	0.00	0.00
S1169_8225	A	G	0.03	0.09	0.57	0.84	0.00	0.00
S11692_839	T	C	0.03	0.09	0.41	0.98	0.00	0.03
S11705_513	T	C	0.21	0.21	-0.04	0.05	0.12	0.03
S1171_32398	T	C	0.23	0.25	0.01	1.00	0.79	0.75
S11728_6032	A	G	0.04	0.07	0.36	0.76	1.00	1.00
S1176_30665	A	G	0.27	0.33	0.12	0.09	0.60	0.84
S11762_6803	A	C	0.10	0.11	0.00	0.52	0.00	0.00
S11772_5134	A	G	0.13	0.15	0.03	1.00	0.88	0.91
S11772_5177	T	G	0.12	0.11	-0.12	0.93	0.94	0.97
S1178_22833	T	C	0.91	0.52	-0.84	0.52	0.50	0.44
S118_99416	A	G	0.01	0.07	0.71	1.00	0.00	0.00
S1181_18715	T	G	0.10	0.10	-0.02	0.25	0.00	0.03
S11844_6950	A	G	0.18	0.16	-0.12	0.00	0.10	0.13
S11844_6968	A	C	0.11	0.10	-0.11	0.84	0.92	0.97
S11846_217	A	G	0.26	0.26	-0.06	0.98	0.85	0.78
S11864_5334	G	C	0.29	0.32	0.04	0.93	0.79	0.91
S119_100849	T	C	0.07	0.11	0.20	0.00	0.10	0.03
S119_100858	G	C	0.26	0.32	0.13	0.25	0.33	0.16
S119_103659	A	G	0.24	0.24	-0.05	0.36	0.10	0.16
S119_103668	A	G	0.47	0.44	-0.12	0.02	0.35	0.34
S11950_314	A	G	0.26	0.22	-0.17	0.00	0.15	0.16
S11950_346	T	C	0.13	0.12	-0.10	0.93	0.92	0.97
S11950_357	T	G	0.11	0.10	-0.09	0.05	0.08	0.06
S11950_362	T	C	0.35	0.29	-0.22	0.98	0.88	0.66
S11958_6080	T	C	0.43	0.42	-0.09	0.89	0.54	0.67
S11958_6530	A	G	0.43	0.45	-0.02	0.14	0.46	0.47
S11960_6889	G	C	0.08	0.11	0.15	0.75	0.94	0.91
S11961_5637	T	C	0.16	0.16	-0.05	0.02	0.13	0.09
S11969_5009	T	C	0.04	0.08	0.46	0.70	1.00	1.00
S1197_27648	A	C	0.14	0.14	-0.04	0.00	0.12	0.03
S12_38415	A	C	0.15	0.13	-0.15	0.86	0.83	0.97
S12_38424	T	G	0.08	0.09	0.02	0.00	1.00	0.00
S120_14183	T	C	0.27	0.33	0.10	0.00	0.46	0.22
S120_41537	A	C	0.16	0.14	-0.16	0.84	0.81	0.97
S120_41539	A	C	0.33	0.22	-0.55	0.11	0.00	0.41
S120_41553	A	C	0.17	0.21	0.08	0.00	0.15	0.16
S12006_56	A	G	0.22	0.18	-0.25	0.80	0.73	0.97
S1203_17943	T	G	0.33	0.34	0.00	0.00	0.31	0.31
S1203_17975	T	G	0.23	0.18	-0.24	0.95	0.92	0.81
S12066_6079	A	T	0.29	0.26	-0.14	0.86	0.79	0.81
S12066_6087	A	G	0.12	0.12	-0.09	0.00	0.00	0.22
S12100_1251	T	C	0.27	0.27	-0.07	0.05	0.15	0.13
S1212_30212	T	C	0.14	0.16	0.06	0.00	0.04	0.09
S1212_30245	A	G	0.82	0.51	-0.71	0.45	0.38	0.41
S1213_36329	T	G	0.15	0.13	-0.16	0.93	0.94	0.84
S12152_6586	A	T	0.07	0.07	-0.03	0.70	1.00	0.97
S1217_37505	G	C	0.10	0.11	0.02	1.00	0.92	0.94
S1217_37512	T	G	0.07	0.10	0.15	0.57	1.00	1.00
S12180_1636	A	C	0.60	0.43	-0.45	0.66	0.71	0.63
S12180_1670	A	G	0.18	0.17	-0.08	0.98	0.94	0.94
S1219_7861	T	C	0.27	0.24	-0.14	0.05	0.19	0.13
S122_6956	A	G	0.10	0.10	-0.07	0.05	0.06	0.06
S122_6962	A	G	0.11	0.11	-0.03	0.77	0.92	1.00
S12204_4764	T	G	0.14	0.13	-0.10	0.05	0.12	0.13

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S1222_731	T	C	0.29	0.45	0.30	0.39	0.67	0.60
S12234_286	T	G	0.30	0.30	-0.04	0.07	0.21	0.25
S12238_1036	T	C	0.07	0.08	0.02	0.02	0.02	0.00
S12238_970	A	G	0.18	0.18	-0.05	0.80	0.88	0.84
S12240_1632	A	G	0.16	0.13	-0.28	1.00	1.00	0.88
S12244_153	A	G	0.47	0.50	0.02	0.48	0.54	0.44
S1227_23131	G	C	0.18	0.17	-0.10	0.00	0.12	0.09
S12295_1012	A	G	0.10	0.13	0.12	0.75	0.00	0.00
S12295_988	T	C	0.17	0.18	0.01	0.05	0.15	0.09
S123_11439	T	C	0.21	0.22	-0.02	0.00	0.15	0.09
S12315_5110	T	G	0.44	0.46	0.00	0.20	0.50	0.28
S12315_5156	T	C	0.46	0.45	-0.07	0.66	0.65	0.75
S12315_5162	T	G	0.16	0.12	-0.26	0.02	0.00	0.06
S12324_620	T	C	0.27	0.29	0.00	0.02	0.21	0.34
S12324_621	T	C	0.29	0.31	0.01	0.98	0.75	0.88
S1235_2972	A	G	0.09	0.09	-0.05	0.16	0.02	0.03
S12385_1158	T	G	0.25	0.31	0.11	0.39	0.15	0.13
S12385_1172	A	G	0.16	0.20	0.10	0.09	0.10	0.09
S12401_1950	A	G	0.18	0.21	0.04	0.05	0.15	0.06
S12430_36	A	G	0.11	0.12	-0.02	0.02	0.04	0.03
S12430_67	A	G	0.30	0.41	0.25	0.23	0.42	0.44
S1247_17460	T	C	0.45	0.44	-0.09	0.00	0.67	0.53
S1248_16092	T	G	0.12	0.10	-0.12	0.95	0.96	0.94
S1248_16124	G	C	0.16	0.12	-0.31	0.07	0.00	0.19
S12515_6437	A	G	0.13	0.13	-0.07	1.00	0.83	0.94
S12543_5388	T	C	0.78	0.49	-0.67	0.82	0.58	0.34
S1255_32680	A	G	0.02	0.09	0.66	0.00	1.00	1.00
S12567_3585	T	C	0.04	0.11	0.39	0.02	1.00	0.91
S12581_2828	A	G	0.18	0.17	-0.14	0.10	0.06	0.19
S12581_2844	A	T	0.31	0.26	-0.21	0.91	0.88	0.78
S1259_412	A	C	0.39	0.49	0.16	0.29	0.52	0.50
S1259_468	A	G	0.16	0.19	0.07	0.95	0.87	0.90
S12590_3235	A	G	0.10	0.10	-0.01	0.50	0.00	0.00
S12590_3280	A	G	0.12	0.13	-0.05	0.00	0.04	0.09
S12680_2254	A	G	0.06	0.11	0.27	0.14	1.00	1.00
S127_102618	T	G	0.33	0.32	-0.08	0.95	0.79	0.69
S127_107915	A	G	0.11	0.10	-0.08	0.23	0.02	0.00
S127_39767	G	C	0.13	0.16	0.09	0.02	0.06	0.06
S1271_26822	A	G	0.05	0.07	0.19	0.80	0.98	1.00
S1271_26863	A	C	0.33	0.33	-0.05	0.02	0.37	0.25
S1271_27081	A	G	0.24	0.25	-0.03	0.00	0.17	0.09
S1272_9096	G	C	0.22	0.22	-0.07	0.02	0.17	0.13
S1272_9126	G	C	0.10	0.10	-0.03	0.00	0.12	0.09
S1272_9135	A	T	0.16	0.15	-0.10	1.00	0.88	0.84
S1272_9215	T	G	0.09	0.10	0.00	0.00	0.10	0.03
S12765_280	T	C	0.21	0.20	-0.10	0.91	0.94	0.97
S12765_314	A	C	0.14	0.12	-0.15	0.05	0.02	0.16
S1277_22158	T	C	0.13	0.11	-0.15	0.93	0.87	0.97
S1277_6896	A	G	0.07	0.09	0.08	0.00	0.06	0.13
S128_32317	T	G	0.03	0.08	0.58	0.00	0.96	1.00
S12810_170	A	G	0.10	0.10	-0.05	0.05	0.04	0.00
S12824_1291	T	C	0.35	0.39	0.06	0.82	0.75	0.72
S12824_1318	A	C	0.11	0.13	0.00	0.95	0.90	1.00
S1284_30975	G	C	0.41	0.43	-0.02	0.98	0.38	0.41
S1284_30990	T	C	0.36	0.38	-0.01	1.00	0.65	0.75
S12848_5326	T	C	0.25	0.28	0.01	0.31	0.04	0.31
S1288_13441	T	G	0.14	0.15	0.01	0.48	0.02	0.09
S1294_23919	A	G	0.16	0.16	-0.07	0.20	0.12	0.16
S1296_37666	A	C	0.12	0.10	-0.22	1.00	1.00	0.88
S12987_1652	T	C	0.17	0.19	-0.01	0.52	0.98	0.91
S13_16991	T	G	0.98	0.53	-0.96	0.50	0.52	0.50
S13_17003	T	C	0.20	0.17	-0.15	0.95	0.98	0.81
S13009_4293	A	C	0.12	0.15	0.09	0.11	0.96	1.00
S13011_1050	T	C	0.11	0.16	0.10	0.18	0.98	0.94
S13012_899	T	C	0.36	0.36	-0.05	0.23	0.31	0.28
S1302_19891	T	G	0.05	0.11	0.34	0.09	0.98	1.00
S1303_37993	A	G	0.08	0.14	0.20	0.93	0.00	0.06
S13037_4348	A	G	0.24	0.22	-0.15	0.09	0.10	0.16
S13037_4910	T	C	0.24	0.22	-0.15	0.07	0.23	0.09
S13037_4942	T	C	0.09	0.08	-0.12	0.18	0.02	0.00
S1309_15560	T	C	0.25	0.24	-0.08	0.00	0.15	0.13
S1309_15567	T	C	0.37	0.38	-0.04	0.00	0.31	0.66

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S1311_23764	T	C	0.13	0.15	0.03	0.23	1.00	0.97
S1313_21808	A	G	0.37	0.38	-0.03	0.09	0.40	0.38
S1313_21841	T	C	0.35	0.35	-0.04	0.32	0.19	0.22
S13137_3006	T	C	0.10	0.09	-0.11	0.16	0.02	0.13
S13160_63	T	C	0.30	0.32	0.01	0.80	0.83	0.91
S13167_4246	A	G	0.26	0.26	-0.07	0.73	0.85	0.88
S1319_17300	A	T	0.09	0.14	0.29	0.70	0.92	0.88
S1319_17324	A	G	0.16	0.29	0.39	0.84	0.67	0.91
S1319_17341	T	C	0.10	0.15	0.29	0.07	0.08	0.09
S13194_6476	A	T	0.04	0.07	0.32	0.75	0.92	1.00
S13194_6501	A	C	0.09	0.10	0.02	0.00	0.06	0.09
S13194_6502	A	G	0.38	0.45	0.08	0.07	0.58	0.31
S13243_1570	A	G	0.09	0.08	-0.12	0.25	0.00	0.09
S13243_1587	A	T	0.09	0.10	0.00	0.02	0.04	0.06
S13258_6512	A	C	0.14	0.15	0.00	0.41	0.04	0.03
S1329_15460	A	G	0.04	0.09	0.35	0.02	1.00	0.97
S1329_32910	A	G	0.10	0.10	-0.09	0.00	0.08	0.03
S133_24486	T	G	0.13	0.20	0.25	0.34	0.90	1.00
S133_24540	G	C	0.09	0.16	0.28	0.00	0.06	0.03
S133_24560	A	G	0.15	0.22	0.27	0.00	0.12	0.19
S13302_587	T	C	0.20	0.20	0.02	0.55	0.10	0.10
S13303_1336	A	G	0.31	0.32	0.02	0.05	0.08	0.63
S1336_17431	T	G	0.13	0.17	0.14	0.20	0.96	0.91
S1341_6380	A	G	0.11	0.11	0.02	0.50	1.00	1.00
S13419_698	T	C	0.07	0.07	-0.07	0.20	0.00	0.00
S1344_38394	A	G	0.08	0.10	0.11	0.43	1.00	1.00
S1348_15734	G	C	0.19	0.25	0.08	1.00	0.94	0.59
S1348_15768	T	C	0.34	0.39	0.07	0.00	0.19	0.25
S135_66151	A	G	0.26	0.39	0.27	0.39	0.21	0.34
S135_66168	G	C	0.08	0.11	0.10	0.00	0.02	0.03
S135_66202	T	G	0.08	0.11	0.15	0.00	0.06	0.03
S1350_14523	A	G	0.03	0.09	0.54	0.07	1.00	1.00
S1350_14570	A	G	0.11	0.11	-0.03	1.00	0.90	1.00
S1351_31001	A	C	0.16	0.12	-0.26	0.83	0.98	0.84
S1351_31038	A	G	0.47	0.49	-0.03	0.75	0.37	0.41
S1351_32208	A	G	0.17	0.17	-0.06	0.00	0.12	0.13
S1351_32228	A	T	0.10	0.11	0.01	1.00	0.94	0.97
S13532_2631	A	G	0.41	0.43	-0.02	1.00	0.46	0.16
S13539_5845	T	C	0.19	0.19	-0.06	0.02	0.13	0.00
S13539_5854	T	C	0.11	0.12	-0.02	0.00	0.06	0.16
S13563_4005	A	G	0.47	0.47	-0.06	0.20	0.42	0.41
S1358_32156	A	T	0.01	0.07	0.72	1.00	0.00	0.00
S136_75184	T	C	0.19	0.21	0.04	1.00	0.90	0.88
S136_75208	A	G	0.12	0.14	0.07	0.02	0.15	0.06
S136_75223	A	G	0.11	0.09	-0.23	0.27	0.00	0.00
S13600_1507	A	G	0.50	0.47	-0.14	0.32	0.58	0.69
S13668_6015	A	G	0.15	0.14	-0.06	0.16	0.00	0.06
S13668_6022	T	G	0.30	0.31	-0.04	0.16	0.25	0.28
S13669_5223	T	G	0.09	0.11	0.00	1.00	0.96	0.94
S1367_31836	A	G	0.12	0.14	0.03	0.95	0.85	0.97
S1368_13358	A	G	0.23	0.30	0.20	0.00	0.38	0.20
S13728_1306	A	T	0.07	0.10	0.13	0.66	1.00	1.00
S13757_3694	T	G	0.15	0.17	0.01	0.98	0.92	0.78
S13757_3703	A	T	0.06	0.07	0.07	0.20	0.00	0.00
S1378_17679	T	G	0.23	0.19	-0.20	0.86	0.90	0.78
S13791_5417	A	G	0.50	0.50	-0.06	0.43	0.54	0.41
S13796_4874	A	T	0.14	0.14	-0.05	0.95	0.92	0.97
S138_52482	G	C	0.01	0.07	0.86	1.00	0.00	0.00
S1380_29469	T	C	0.11	0.10	-0.08	0.00	0.08	0.03
S1386_5636	A	G	0.04	0.10	0.43	0.45	0.00	0.00
S13861_454	T	G	0.16	0.17	-0.04	0.11	0.13	0.13
S13861_465	T	C	0.31	0.36	0.07	0.82	0.73	0.83
S1387_36537	A	G	0.07	0.12	0.20	0.09	0.98	0.97
S1388_19900	T	G	0.40	0.44	0.03	0.86	0.52	0.66
S139_12976	A	G	0.17	0.16	-0.09	1.00	0.81	0.94
S139_13129	A	G	0.28	0.24	-0.19	0.02	0.23	0.13
S139_13155	T	C	0.12	0.09	-0.22	0.70	0.98	1.00
S1390_22673	T	C	0.11	0.10	-0.09	1.00	0.92	0.94
S1390_22700	G	C	0.11	0.11	-0.05	0.00	0.06	0.09
S13904_1400	T	C	0.21	0.20	-0.08	0.00	0.08	0.09

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S1391_7052	G	C	0.83	0.51	-0.73	0.34	0.44	0.38
S1391_7058	A	G	0.97	0.53	-0.94	0.59	0.52	0.50
S1391_7065	T	C	0.15	0.14	-0.10	0.14	0.08	0.00
S1391_7070	T	C	0.11	0.10	-0.11	0.00	0.04	0.09
S1391_7076	T	C	0.12	0.11	-0.10	0.00	0.04	0.09
S13917_5417	A	T	0.11	0.09	-0.11	0.31	0.00	0.03
S13917_5468	T	C	0.10	0.09	-0.11	0.00	0.17	0.00
S13999_3069	A	G	0.36	0.30	-0.24	0.00	0.21	0.31
S14_133964	T	C	0.09	0.12	0.27	0.75	0.04	0.00
S14_87193	T	C	0.13	0.14	0.04	0.98	0.92	0.94
S140_58783	A	T	0.30	0.38	0.14	0.39	0.19	0.19
S140_58789	A	T	0.16	0.18	0.04	0.34	0.06	0.16
S140_8896	T	C	0.19	0.19	-0.06	0.98	0.87	0.93
S140_8908	G	C	0.11	0.12	0.00	0.98	0.90	0.94
S140_8912	A	G	0.15	0.15	-0.05	0.98	0.90	0.94
S140_98162	A	G	0.32	0.34	-0.01	0.00	0.27	0.31
S14034_5700	A	G	0.13	0.15	0.03	0.05	0.08	0.06
S1405_2955	G	C	0.03	0.08	0.57	1.00	0.00	0.00
S14050_5850	T	C	0.19	0.18	-0.06	0.02	0.15	0.03
S14050_6014	A	G	0.26	0.32	0.12	0.16	0.87	0.81
S1406_13321	T	C	0.37	0.45	0.12	0.66	0.63	0.41
S1406_7525	G	C	0.10	0.10	-0.05	0.00	0.10	0.10
S14122_5056	A	G	0.33	0.38	0.05	0.05	0.19	0.28
S14122_5100	T	C	0.12	0.13	0.00	0.07	0.12	0.03
S14122_5989	T	G	0.25	0.22	-0.14	0.82	0.90	0.84
S1413_28484	T	C	0.11	0.13	0.05	1.00	0.94	0.84
S14148_5398	T	C	0.25	0.26	-0.03	0.02	0.13	0.22
S1417_28038	T	C	0.07	0.12	0.24	0.09	1.00	0.97
S14173_5361	A	C	0.39	0.48	0.15	0.25	0.56	0.63
S142_57083	T	G	0.12	0.11	-0.12	0.93	0.88	1.00
S142_57084	A	G	0.28	0.29	-0.04	0.00	0.13	0.13
S142_57099	A	G	0.12	0.13	0.00	0.98	0.88	0.97
S142_7705	A	G	0.16	0.16	0.00	0.09	0.04	0.10
S142_7715	A	T	0.12	0.12	-0.03	1.00	0.96	0.90
S142_7716	A	G	0.28	0.31	0.03	0.84	0.75	0.77
S1420_23317	A	G	0.18	0.17	-0.10	0.02	0.17	0.06
S1420_4848	T	C	0.38	0.39	-0.05	0.45	0.27	0.22
S1424_21317	G	C	0.07	0.08	0.01	0.14	0.02	0.00
S1425_13069	T	C	0.09	0.10	0.02	0.00	0.10	0.09
S1429_30415	A	C	0.03	0.09	0.52	0.05	1.00	1.00
S1429_30439	T	C	0.15	0.17	0.05	1.00	1.00	0.94
S143_103228	G	C	0.29	0.29	-0.05	0.09	0.10	0.31
S143_46896	A	G	0.15	0.15	-0.07	0.00	0.12	0.03
S143_94237	A	C	0.50	0.28	-0.83	0.59	0.50	0.97
S143_94279	A	T	0.12	0.12	-0.03	0.00	0.06	0.09
S1432_7969	A	C	0.38	0.44	0.07	0.05	0.42	0.41
S1432_7978	T	C	0.29	0.32	0.02	0.05	0.17	0.28
S1432_7981	A	C	0.08	0.10	0.10	0.02	0.04	0.13
S1440_33057	A	G	0.16	0.18	0.04	0.00	0.04	0.03
S1440_33068	T	G	0.09	0.10	0.06	0.55	0.00	0.00
S14446_1234	T	G	0.34	0.28	-0.23	0.00	0.25	0.23
S14446_1236	A	T	0.34	0.27	-0.26	1.00	0.67	0.90
S14446_1246	A	C	0.33	0.25	-0.26	0.39	0.15	0.03
S145_69530	A	G	0.24	0.24	-0.03	0.00	0.17	0.09
S14507_1023	T	C	0.15	0.18	0.07	0.07	0.10	0.13
S1456_6099	A	G	0.18	0.16	-0.12	0.00	0.17	0.13
S14580_5669	T	C	0.25	0.28	0.03	0.80	0.04	0.19
S14580_5685	T	G	0.18	0.15	-0.26	0.98	1.00	0.84
S1463_20040	T	G	0.09	0.11	0.12	0.00	0.17	0.09
S1463_9727	T	G	0.18	0.19	-0.07	0.02	0.13	0.09
S14632_3524	A	C	0.24	0.22	-0.08	0.00	0.29	0.00
S1467_16733	A	C	0.32	0.32	-0.06	0.07	0.23	0.22
S1467_16754	T	C	0.12	0.12	-0.02	0.00	0.02	0.09
S1467_16771	A	G	0.10	0.12	0.10	0.38	0.00	0.03
S1471_20193	A	C	0.19	0.15	-0.26	0.09	0.00	0.25
S1471_20243	A	G	0.18	0.20	0.02	0.86	0.96	1.00
S14749_3705	T	C	0.22	0.24	-0.01	0.84	0.83	0.88
S14758_5421	T	G	0.13	0.17	0.10	0.05	0.06	0.17
S14758_5431	A	G	0.16	0.19	0.10	0.05	0.08	0.03
S14758_5445	A	T	0.35	0.41	0.08	0.77	0.62	0.67

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S14758_5452	T	C	0.06	0.08	0.15	0.16	0.04	0.03
S14786_1514	T	C	0.04	0.10	0.51	0.18	1.00	1.00
S14791_4725	A	C	0.12	0.12	-0.04	0.02	0.06	0.09
S1481_25665	T	C	0.12	0.12	-0.03	0.05	0.02	0.06
S1481_25688	T	G	0.15	0.12	-0.18	0.98	1.00	0.94
S14845_2087	G	C	0.46	0.35	-0.36	0.91	0.81	0.59
S1485_19195	T	G	0.10	0.13	0.14	0.73	0.02	0.03
S14908_4660	T	C	0.19	0.28	0.24	0.86	0.15	0.28
S14908_4731	A	G	0.11	0.11	-0.05	0.00	0.12	0.06
S14922_1650	T	G	0.10	0.11	0.05	0.05	0.06	0.09
S14922_1655	A	T	0.13	0.13	-0.03	0.95	0.98	0.97
S14922_1658	A	G	0.11	0.11	-0.07	0.00	0.04	0.06
S1493_19783	G	C	0.16	0.15	-0.09	0.84	0.90	0.94
S1496_7941	A	G	0.27	0.33	0.09	0.00	0.33	0.06
S15_125575	A	T	0.13	0.13	-0.06	1.00	0.96	0.97
S15_125615	A	G	0.10	0.11	-0.07	0.00	0.06	0.03
S15_125619	T	G	0.15	0.15	-0.06	0.00	0.06	0.00
S15_125656	A	G	0.12	0.14	0.03	1.00	0.88	0.75
S15_184193	A	C	0.03	0.09	0.56	0.11	1.00	0.97
S1505_4120	G	C	0.07	0.09	0.07	0.00	0.12	0.00
S15082_4786	T	C	0.13	0.15	0.04	0.90	0.96	0.83
S15082_4799	A	T	0.14	0.15	-0.01	0.10	0.04	0.09
S15154_2511	A	G	0.38	0.36	-0.11	0.93	0.69	0.84
S15154_2543	A	G	0.29	0.30	-0.05	0.93	0.75	0.88
S15179_120	T	C	0.40	0.43	0.01	0.18	0.33	0.41
S1519_13669	T	G	0.07	0.09	0.10	0.70	1.00	1.00
S1520_628	T	C	0.35	0.48	0.21	0.65	0.62	0.50
S15236_2793	A	T	0.05	0.11	0.44	0.66	0.00	0.00
S15236_2826	T	G	0.11	0.12	-0.02	1.00	0.94	0.94
S1524_11439	A	C	0.11	0.14	0.09	0.80	0.06	0.03
S1524_29139	T	C	0.15	0.16	0.01	0.07	0.14	0.07
S1524_34591	A	C	0.25	0.26	-0.04	0.02	0.19	0.09
S15269_4165	T	C	0.21	0.18	-0.21	0.00	0.17	0.00
S15269_4174	T	C	0.17	0.14	-0.14	0.00	0.15	0.06
S15269_4192	A	G	0.86	0.48	-0.81	0.89	0.50	0.50
S15269_4223	A	G	0.18	0.16	-0.12	0.98	0.87	0.88
S153_73591	A	T	0.02	0.08	0.56	1.00	0.00	0.00
S15300_4750	A	G	0.21	0.19	-0.15	0.05	0.10	0.16
S15312_242	A	G	0.78	0.50	-0.63	0.39	0.54	0.56
S15361_1717	A	G	0.10	0.10	-0.06	0.07	0.02	0.22
S15361_1740	T	C	0.22	0.19	-0.15	0.77	0.92	0.84
S15379_3150	T	C	0.74	0.47	-0.61	0.11	0.33	0.41
S15379_4609	A	G	0.15	0.13	-0.12	0.98	0.88	1.00
S15379_4621	T	C	0.14	0.13	-0.10	0.00	0.06	0.06
S15422_677	A	G	0.16	0.15	-0.13	0.16	0.06	0.06
S15422_711	T	G	0.12	0.11	-0.12	0.00	0.12	0.06
S15422_717	T	C	0.84	0.51	-0.74	0.73	0.37	0.44
S15429_600	A	G	0.12	0.11	-0.09	0.03	0.04	0.16
S1546_13977	T	C	0.28	0.27	-0.12	0.00	0.23	0.16
S1546_14018	A	C	0.08	0.08	0.01	0.75	1.00	1.00
S1548_1972	T	C	0.46	0.27	-0.73	0.66	0.52	0.97
S155_96501	A	C	0.07	0.08	0.09	0.25	0.00	0.00
S15549_2547	A	C	0.12	0.10	-0.20	0.05	0.00	0.09
S15549_2554	T	C	0.14	0.16	0.07	0.00	0.10	0.06
S15549_2593	A	G	0.08	0.11	0.20	0.98	0.88	0.97
S1555_20885	A	G	0.02	0.08	0.63	1.00	0.00	0.00
S15570_1559	G	C	0.11	0.13	0.06	0.93	0.94	0.93
S156_148	T	G	0.18	0.20	0.04	0.00	0.17	0.16
S156_23239	T	C	0.47	0.46	-0.10	0.23	0.46	0.38
S156_71938	A	G	0.17	0.17	-0.06	1.00	0.87	0.84
S15603_2286	A	G	0.19	0.17	-0.16	0.00	0.15	0.10
S1567_28880	A	G	0.36	0.41	0.09	0.00	0.37	0.47
S15670_258	A	G	0.17	0.13	-0.30	0.00	0.15	0.00
S1568_27410	A	G	0.09	0.13	0.15	0.82	0.00	0.06
S1568_27415	T	G	0.11	0.10	-0.13	0.95	0.98	0.97
S1569_11063	T	C	0.12	0.12	-0.02	0.11	0.04	0.03
S1569_21290	A	G	0.06	0.08	0.15	0.21	0.00	0.00
S1572_25829	A	G	0.24	0.25	-0.04	1.00	0.69	0.80
S15723_650	A	G	0.44	0.45	-0.04	0.18	0.37	0.34

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S15723_667	T	C	0.10	0.10	-0.03	0.98	0.88	0.91
S15724_1425	T	C	0.11	0.14	0.09	0.05	0.12	0.09
S15724_1659	A	C	0.15	0.17	0.05	0.57	0.06	0.00
S15724_1728	G	C	0.19	0.17	-0.13	0.11	0.08	0.13
S15797_3886	G	C	0.12	0.17	0.13	0.86	0.06	0.09
S158_26351	A	C	0.11	0.12	-0.04	0.10	0.20	0.03
S15820_1568	T	G	0.13	0.11	-0.21	0.95	1.00	0.78
S15820_1572	A	C	0.78	0.48	-0.68	0.59	0.54	0.56
S15820_1582	A	G	0.14	0.14	-0.05	0.98	0.92	0.88
S15834_1114	A	G	0.74	0.47	-0.62	0.48	0.38	0.31
S15834_1122	T	C	0.46	0.35	-0.34	1.00	0.71	0.63
S15871_2247	T	C	0.10	0.10	-0.10	0.95	0.96	0.87
S15871_3703	T	C	0.75	0.49	-0.60	0.70	0.37	0.44
S15899_454	T	C	0.48	0.44	-0.15	0.71	0.75	0.75
S15900_1429	T	C	0.19	0.23	0.10	0.14	0.08	0.09
S1593_31180	A	G	0.17	0.19	0.02	0.00	0.15	0.06
S1593_31227	A	T	0.22	0.23	-0.01	0.05	0.08	0.19
S16000_3753	A	T	0.11	0.11	-0.04	0.00	0.06	0.03
S16016_2646	T	C	0.24	0.23	-0.13	0.95	0.88	0.81
S16046_3016	T	C	0.11	0.11	-0.01	0.00	0.06	0.03
S16053_1269	T	C	0.13	0.15	0.03	0.10	0.13	0.00
S16053_1288	T	G	0.30	0.36	0.12	0.07	0.23	0.47
S16106_276	T	C	0.15	0.13	-0.13	1.00	0.84	1.00
S16106_280	T	C	0.13	0.12	-0.10	0.09	0.10	0.00
S16106_294	A	T	0.10	0.09	-0.10	0.02	0.08	0.03
S16106_298	A	G	0.21	0.19	-0.15	0.00	0.10	0.16
S16106_299	A	T	0.13	0.12	-0.10	0.02	0.08	0.13
S1614_4976	T	G	0.11	0.11	-0.04	0.00	0.12	0.03
S16155_1530	A	C	0.19	0.15	-0.28	0.09	0.02	0.25
S162_91561	A	G	0.03	0.09	0.45	0.93	0.00	0.00
S16209_1107	T	C	0.11	0.15	0.14	0.16	0.96	0.97
S1623_221	A	G	0.05	0.09	0.31	0.05	1.00	1.00
S16237_2269	A	G	0.14	0.13	-0.10	0.00	0.12	0.03
S16237_2280	A	G	0.10	0.14	0.16	0.98	0.02	0.03
S16250_2482	A	G	0.32	0.37	0.07	0.36	0.17	0.19
S1627_12176	A	G	0.82	0.50	-0.71	0.55	0.37	0.41
S16299_103	A	G	0.11	0.11	-0.05	0.00	0.08	0.09
S1631_1371	A	G	0.17	0.16	-0.12	0.00	0.19	0.22
S16313_2365	T	G	0.12	0.11	-0.14	0.02	0.00	0.07
S16313_2372	A	G	0.23	0.31	0.16	1.00	0.74	0.70
S16317_1072	T	G	0.28	0.21	-0.26	1.00	0.96	0.81
S16348_658	A	T	0.12	0.11	-0.11	0.00	0.12	0.13
S16348_671	T	G	0.15	0.14	-0.11	1.00	0.88	0.94
S16358_3427	G	C	0.14	0.13	-0.05	0.05	0.06	0.03
S1636_1759	T	C	0.18	0.16	-0.13	0.07	0.10	0.25
S1636_1770	A	G	0.17	0.15	-0.11	0.02	0.10	0.13
S1636_1771	G	C	0.11	0.11	-0.07	1.00	0.90	0.94
S1636_27002	A	G	0.21	0.20	-0.09	0.05	0.12	0.16
S16367_1147	T	C	0.12	0.16	0.10	0.89	0.98	0.97
S16392_2133	T	G	0.06	0.10	0.26	0.15	0.04	0.00
S164_75994	A	G	0.35	0.30	-0.21	0.14	0.15	0.16
S164_76023	T	C	0.84	0.49	-0.78	0.88	0.50	0.53
S164_76025	T	C	0.11	0.10	-0.11	0.14	0.00	0.13
S1643_23107	T	C	0.06	0.11	0.26	0.09	1.00	1.00
S16441_2781	T	C	0.03	0.09	0.40	0.98	0.00	0.00
S16467_1237	G	C	0.94	0.52	-0.90	0.59	0.62	0.50
S16467_3173	A	T	0.13	0.11	-0.13	0.27	0.02	0.06
S1652_6732	A	G	0.12	0.12	-0.09	0.09	0.06	0.06
S1652_6744	A	G	0.12	0.11	-0.13	0.18	0.00	0.13
S1652_6760	T	C	0.34	0.29	-0.21	0.89	0.73	0.88
S16521_1400	T	C	0.10	0.10	-0.02	0.43	0.02	0.06
S16582_3108	T	C	0.08	0.08	-0.06	0.27	0.02	0.06
S1661_1344	A	G	0.10	0.11	0.01	0.00	0.12	0.13
S1661_1355	A	C	0.12	0.16	0.15	0.07	0.16	0.16
S1661_1360	T	C	0.10	0.12	0.06	0.00	0.04	0.06
S16610_2603	A	G	0.51	0.38	-0.37	0.07	0.21	0.22
S16610_2629	A	C	0.13	0.12	-0.11	0.09	0.04	0.09
S16610_2664	A	G	0.13	0.12	-0.11	0.07	0.15	0.03
S16610_2683	T	C	0.50	0.38	-0.36	0.84	0.71	0.72

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S16610_2707	G	C	0.18	0.16	-0.14	0.98	0.81	0.97
S16643_485	A	G	0.13	0.12	-0.12	0.00	0.04	0.16
S16643_504	A	G	0.12	0.11	-0.14	0.64	1.00	0.97
S16643_517	A	G	0.14	0.12	-0.12	0.07	0.00	0.19
S16660_685	G	C	0.04	0.09	0.44	0.58	0.00	0.00
S1668_13073	A	G	0.44	0.42	-0.10	0.80	0.81	0.81
S167_50906	G	C	0.09	0.10	0.04	0.00	0.06	0.13
S167_76090	T	G	0.03	0.09	0.60	1.00	0.00	0.00
S16750_2280	A	T	0.20	0.21	-0.01	0.11	0.08	0.16
S16792_2794	A	C	0.09	0.10	-0.04	0.02	0.06	0.00
S16792_2801	T	G	0.15	0.15	-0.05	0.02	0.10	0.09
S16792_2809	G	C	0.17	0.19	0.03	0.11	0.15	0.13
S16792_2893	A	G	0.08	0.10	0.10	0.48	0.00	0.00
S1685_18279	T	C	0.16	0.16	-0.02	0.86	0.88	0.91
S1685_18282	T	C	0.10	0.10	-0.04	0.73	0.98	1.00
S16860_3100	A	T	0.10	0.09	-0.12	0.30	0.00	0.00
S16860_3101	T	C	0.11	0.11	-0.04	0.02	0.10	0.13
S16891_692	G	C	0.07	0.08	0.00	0.23	0.02	0.03
S169_51326	T	G	0.50	0.28	-0.81	0.57	0.54	0.97
S16909_3391	G	C	0.44	0.41	-0.15	0.02	0.75	0.78
S1694_4274	G	C	0.13	0.12	-0.13	0.98	1.00	0.97
S16983_423	A	G	0.28	0.28	-0.05	0.80	0.79	0.78
S16983_477	A	G	0.12	0.12	-0.05	0.00	0.08	0.03
S17_159436	A	C	0.07	0.10	0.30	0.34	1.00	1.00
S17_33299	T	C	0.02	0.12	0.82	0.00	0.02	0.13
S17009_2564	A	G	0.14	0.15	0.03	0.09	0.13	0.06
S1701_6968	A	G	0.08	0.12	0.15	0.02	1.00	0.94
S17027_3412	A	C	0.33	0.38	0.07	0.07	0.40	0.03
S17082_1285	T	G	0.10	0.10	-0.07	0.00	0.06	0.00
S17082_1328	A	G	0.25	0.40	0.33	0.30	0.27	0.53
S17093_1387	G	C	0.43	0.49	0.06	0.73	0.38	0.53
S1713_23922	T	C	0.09	0.10	0.01	0.07	0.06	0.09
S1713_23959	T	G	0.39	0.25	-0.46	0.86	0.96	0.59
S17144_643	T	C	0.01	0.08	0.71	1.00	0.00	0.00
S17167_1499	T	C	0.10	0.11	-0.03	0.00	0.12	0.03
S17167_1532	A	T	0.12	0.14	0.02	0.93	0.96	0.91
S17169_2169	A	G	0.17	0.20	0.08	0.36	0.96	0.91
S1720_29800	T	C	0.18	0.23	0.11	0.93	0.10	0.13
S17236_1436	A	C	0.14	0.13	-0.13	0.91	0.96	0.94
S17236_1441	T	G	0.20	0.16	-0.29	0.05	0.00	0.19
S1726_26831	T	C	0.31	0.40	0.19	0.00	0.63	0.78
S1727_29708	T	C	0.07	0.08	-0.02	0.20	0.02	0.06
S1728_28125	A	C	0.13	0.14	0.00	0.98	0.92	0.97
S1728_28131	A	G	0.39	0.43	0.05	0.20	0.48	0.34
S173_77729	G	C	0.28	0.30	0.00	0.98	0.56	0.81
S17357_2855	A	G	0.08	0.11	0.07	1.00	0.96	0.88
S1736_24812	A	G	0.06	0.09	0.28	0.86	0.00	0.00
S17379_2064	A	G	0.34	0.33	-0.10	0.93	0.77	0.75
S1740_19632	A	C	0.13	0.13	-0.04	0.00	0.12	0.03
S1740_19636	A	C	0.10	0.14	0.13	0.89	0.06	0.06
S17409_332	A	G	0.31	0.26	-0.22	0.00	0.17	0.13
S17409_353	T	C	0.12	0.11	-0.09	0.05	0.12	0.09
S17409_385	A	T	0.34	0.29	-0.23	0.97	0.79	0.81
S1750_24297	T	C	0.17	0.18	-0.01	0.93	0.85	0.91
S1751_27526	T	C	0.22	0.21	-0.07	0.02	0.15	0.13
S1751_27533	G	C	0.21	0.19	-0.14	0.89	0.90	0.88
S17540_1007	A	T	0.10	0.11	0.03	0.02	0.04	0.13
S17540_931	G	C	0.05	0.10	0.29	0.07	0.02	0.13
S17540_961	T	C	0.08	0.13	0.30	0.45	0.96	0.97
S176_79847	T	C	0.03	0.09	0.44	0.05	1.00	0.97
S17609_678	A	G	0.29	0.35	0.10	0.66	0.77	0.80
S17620_1122	T	G	0.10	0.10	-0.07	0.98	0.92	0.84
S1763_24252	T	C	0.11	0.12	-0.04	0.02	0.08	0.19
S17638_490	A	G	0.09	0.10	0.12	0.59	0.00	0.00
S17648_480	A	C	0.20	0.16	-0.27	0.98	1.00	0.84
S1765_31377	A	C	0.48	0.48	-0.04	0.77	0.42	0.53
S17722_2372	T	C	0.11	0.12	0.03	1.00	0.94	0.88
S17728_2815	G	C	0.17	0.18	0.01	0.55	0.90	0.97
S17728_2908	A	T	0.11	0.12	-0.07	0.93	0.90	0.94

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S1778_13728	T	G	0.06	0.09	0.22	0.00	0.06	0.00
S1778_13761	G	C	0.06	0.10	0.26	1.00	1.00	0.89
S178_3229	A	G	0.05	0.09	0.38	0.20	0.00	0.00
S17805_2422	T	C	0.06	0.11	0.38	0.23	0.00	0.00
S17829_1456	T	C	0.06	0.08	0.20	0.50	0.00	0.00
S17829_1492	A	G	0.13	0.15	0.10	1.00	0.90	0.93
S1785_14837	A	G	0.45	0.44	-0.06	0.02	0.40	0.34
S17857_2281	T	C	0.06	0.11	0.28	0.86	0.00	0.00
S17880_2925	A	G	0.29	0.25	-0.19	0.93	0.85	0.84
S17880_2941	A	T	0.33	0.23	-0.47	0.80	0.62	0.97
S17883_2760	T	C	0.10	0.11	0.03	0.00	0.12	0.06
S17883_2790	G	C	0.18	0.23	0.07	0.00	0.21	0.16
S17904_1551	T	G	0.06	0.11	0.33	0.07	0.04	0.10
S17936_2409	A	T	0.47	0.48	-0.02	0.74	0.60	0.50
S1799_22447	T	C	0.02	0.08	0.68	0.98	0.00	0.00
S18_76919	A	T	0.02	0.07	0.58	0.00	1.00	1.00
S180_32736	G	C	0.13	0.12	-0.09	0.95	0.88	0.94
S180_32847	A	G	0.09	0.08	-0.12	0.68	1.00	0.97
S1803_25171	G	C	0.17	0.20	0.05	0.00	0.15	0.22
S1803_25192	T	C	0.08	0.09	0.01	0.52	0.00	0.00
S1804_405	A	C	0.15	0.15	-0.09	0.09	0.04	0.19
S18083_650	A	C	0.13	0.14	0.02	0.95	0.94	0.94
S18083_658	G	C	0.39	0.42	0.04	0.25	0.29	0.19
S181_67410	T	C	0.21	0.27	0.16	0.05	0.23	0.27
S181_67414	T	G	0.16	0.13	-0.17	0.02	0.00	0.20
S181_67423	A	C	0.11	0.10	-0.11	0.93	0.94	0.93
S181_67425	A	G	0.18	0.22	0.05	0.02	0.17	0.20
S18117_1838	A	G	0.11	0.13	0.13	0.50	0.00	0.13
S1814_11936	T	C	0.18	0.16	-0.13	0.00	0.10	0.09
S1816_1019	T	G	0.02	0.08	0.60	1.00	0.02	0.00
S1819_13909	A	G	0.36	0.40	0.08	0.00	0.35	0.41
S18191_434	G	C	0.19	0.20	0.02	0.00	0.15	0.09
S18219_2878	A	G	0.08	0.11	0.08	0.02	0.12	0.06
S18236_2224	A	G	0.17	0.19	0.02	0.07	0.15	0.03
S18261_1346	T	C	0.07	0.10	0.11	0.07	0.04	0.06
S18261_1354	A	G	0.28	0.34	0.10	0.75	0.75	0.75
S18262_2490	A	G	0.13	0.12	-0.09	0.02	0.04	0.09
S1828_24457	G	C	0.38	0.31	-0.24	0.84	0.92	0.66
S1829_2783	A	G	0.16	0.19	0.01	1.00	0.90	0.81
S18349_2243	T	C	0.20	0.22	0.05	0.09	0.13	0.09
S18372_1816	T	C	0.13	0.10	-0.27	0.11	0.00	0.16
S18372_1824	G	C	0.16	0.17	0.02	0.95	0.92	0.91
S184_80842	A	G	0.02	0.09	0.53	0.98	0.02	0.00
S1840_10749	A	T	0.04	0.09	0.45	0.09	1.00	1.00
S18446_1126	A	G	0.17	0.14	-0.20	0.86	0.75	0.97
S185_68866	A	G	0.20	0.20	-0.05	1.00	0.81	0.88
S1850_22581	T	G	0.18	0.16	-0.13	0.05	0.06	0.09
S1850_22583	A	G	0.10	0.10	-0.06	0.02	0.12	0.03
S1852_10832	T	C	0.29	0.29	-0.06	0.07	0.21	0.09
S1852_11694	A	G	0.39	0.37	-0.10	0.16	0.31	0.34
S1852_6672	T	C	0.09	0.09	-0.04	0.18	0.02	0.00
S1853_22744	T	C	0.06	0.11	0.26	0.07	0.98	1.00
S18546_1180	T	C	0.26	0.21	-0.24	0.64	1.00	0.81
S18546_1206	T	G	0.14	0.14	-0.03	0.00	0.16	0.04
S1855_18882	T	C	0.09	0.15	0.22	0.95	0.00	0.03
S18600_2694	A	G	0.14	0.13	-0.12	0.00	0.17	0.03
S18600_2720	T	C	0.08	0.08	-0.05	0.27	0.00	0.00
S18600_2727	A	G	0.10	0.09	-0.11	0.02	0.08	0.06
S18607_1504	G	C	0.14	0.16	0.00	0.02	0.06	0.13
S18635_2831	T	C	0.13	0.13	-0.03	0.09	0.10	0.06
S18635_2848	A	G	0.08	0.09	0.03	0.98	0.94	0.97
S1868_24200	T	C	0.39	0.42	0.02	0.98	0.56	0.53
S18712_347	T	C	0.24	0.23	-0.06	0.05	0.31	0.03
S1873_10804	A	G	0.64	0.45	-0.49	0.18	0.35	0.31
S1873_10806	T	C	0.49	0.37	-0.36	0.64	0.79	0.75
S1873_10815	A	G	0.76	0.48	-0.64	0.57	0.65	0.66
S1873_10817	A	G	0.58	0.41	-0.44	0.24	0.32	0.41
S1873_10818	T	G	0.25	0.21	-0.17	0.02	0.08	0.16
S1873_10819	T	C	0.18	0.16	-0.13	1.00	0.92	0.88

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S18730_2629	A	G	0.14	0.14	-0.08	1.00	0.92	1.00
S18730_2632	A	T	0.09	0.08	-0.07	0.32	0.00	0.00
S1875_11117	A	G	0.40	0.41	-0.02	0.05	0.42	0.47
S1876_24497	A	G	0.43	0.44	-0.04	0.70	0.67	0.44
S18788_1032	A	G	0.12	0.12	-0.05	0.07	0.12	0.03
S18788_424	T	C	0.33	0.34	-0.05	0.23	0.21	0.22
S188_16191	T	G	0.14	0.17	0.07	0.98	0.90	0.97
S188_16213	A	G	0.10	0.11	0.03	0.07	0.04	0.06
S1884_23158	T	C	0.17	0.19	-0.02	0.05	0.10	0.16
S1887_25536	A	T	0.17	0.16	-0.13	1.00	0.81	0.97
S18878_2311	A	C	0.16	0.18	0.05	0.98	0.96	0.94
S189_49961	T	C	0.23	0.36	0.28	1.00	0.27	0.22
S189_50036	A	C	0.44	0.34	-0.31	0.82	0.75	0.81
S189_50053	A	G	0.14	0.13	-0.11	0.00	0.06	0.09
S189_50070	T	C	0.30	0.25	-0.21	0.00	0.23	0.09
S18903_2101	T	C	0.11	0.10	-0.07	0.00	0.04	0.03
S1894_23326	G	C	0.04	0.07	0.24	0.26	0.02	0.03
S19_74531	T	C	0.02	0.07	0.64	0.00	1.00	1.00
S1901_29836	A	T	0.15	0.15	-0.04	0.00	0.19	0.00
S19069_1341	T	G	0.55	0.39	-0.43	0.75	0.77	0.63
S19137_789	A	G	0.10	0.12	0.05	0.00	0.04	0.06
S19137_797	A	T	0.10	0.11	0.00	0.71	0.94	0.97
S1916_11557	T	C	0.14	0.13	-0.11	0.05	0.13	0.03
S1916_11561	A	T	0.16	0.14	-0.13	0.02	0.15	0.03
S1916_11593	A	G	0.22	0.19	-0.16	0.11	0.13	0.09
S1916_11594	A	T	0.12	0.12	-0.11	1.00	0.90	0.94
S1916_11596	T	C	0.89	0.50	-0.84	0.80	0.52	0.53
S1916_11605	A	G	0.82	0.50	-0.71	0.50	0.42	0.41
S19194_97	A	G	0.14	0.17	0.00	1.00	0.88	0.94
S19205_2670	T	C	0.20	0.18	-0.13	0.00	0.12	0.06
S19209_2052	A	G	0.41	0.42	0.00	0.16	0.38	0.31
S19210_2344	A	T	0.02	0.07	0.66	0.00	1.00	1.00
S1922_13339	A	C	0.05	0.10	0.28	0.07	0.98	1.00
S19222_1895	T	C	0.14	0.16	0.06	0.86	0.90	0.84
S193_65630	A	T	0.08	0.10	0.14	0.00	0.10	0.09
S1931_28741	A	G	0.38	0.47	0.13	0.80	0.50	0.40
S1931_28759	A	T	0.39	0.44	0.06	0.59	0.65	0.77
S1936_17876	A	G	0.13	0.17	0.12	0.86	0.04	0.06
S19386_2242	A	G	0.38	0.37	-0.09	0.02	0.85	0.63
S1940_23510	T	C	0.17	0.21	0.08	0.45	0.96	0.81
S1940_23583	A	T	0.29	0.33	0.06	0.16	0.17	0.22
S1944_24675	T	C	0.10	0.11	0.01	0.00	0.08	0.06
S1944_30744	A	G	0.11	0.10	-0.09	0.02	0.12	0.03
S1944_30748	T	C	0.12	0.09	-0.26	0.70	0.98	1.00
S1947_11336	A	G	0.03	0.10	0.49	0.00	1.00	1.00
S19610_841	T	C	0.20	0.21	0.02	0.66	0.92	0.91
S19650_313	T	C	0.34	0.44	0.19	0.48	0.77	0.63
S19666_1825	A	G	0.08	0.11	0.15	0.59	0.00	0.03
S19666_1827	A	C	0.15	0.16	0.00	0.00	0.13	0.16
S1967_26342	T	C	0.08	0.10	0.11	0.07	0.10	0.03
S1967_26346	A	T	0.10	0.12	0.06	0.05	0.02	0.03
S1967_7209	T	C	0.10	0.10	-0.04	0.05	0.08	0.06
S1967_7212	T	G	0.24	0.24	-0.05	0.89	0.83	0.81
S19728_1272	A	G	0.15	0.16	0.01	0.82	0.88	0.94
S19728_1284	A	T	0.15	0.17	0.02	0.77	0.92	0.91
S19728_1294	A	G	0.13	0.14	-0.02	0.00	0.08	0.09
S19742_1286	T	C	0.43	0.44	-0.04	0.41	0.35	0.34
S19742_941	T	G	0.14	0.14	-0.04	0.27	0.02	0.03
S1981_11982	A	G	0.12	0.13	0.00	0.73	0.92	1.00
S19840_894	A	C	0.15	0.18	0.04	0.95	0.77	0.90
S19840_898	T	C	0.14	0.15	0.01	0.02	0.12	0.13
S19840_899	A	C	0.08	0.10	0.08	0.11	0.02	0.03
S19867_1329	G	C	0.45	0.48	0.02	0.50	0.48	0.34
S19891_2221	T	G	0.23	0.24	-0.03	0.98	0.85	0.69
S1990_1319	T	C	0.13	0.13	-0.06	0.00	0.10	0.03
S19934_1960	A	G	0.29	0.32	0.02	0.98	0.81	0.63
S1995_2100	A	T	0.18	0.19	-0.02	0.00	0.10	0.03
S19965_2007	A	G	0.22	0.23	-0.02	0.05	0.13	0.25
S1997_29831	T	C	0.20	0.20	-0.04	0.02	0.08	0.19

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S2_111902	A	T	0.24	0.21	-0.17	0.93	0.88	0.78
S2_111911	T	G	0.45	0.36	-0.30	0.11	0.24	0.25
S2_111935	A	C	0.16	0.15	-0.12	0.05	0.06	0.07
S2_111955	A	C	0.19	0.17	-0.13	0.00	0.10	0.16
S2005_26251	A	C	0.41	0.39	-0.11	0.00	0.31	0.28
S2006_1231	A	G	0.31	0.30	-0.06	0.91	0.87	0.72
S2009_20005	A	C	0.13	0.13	-0.03	0.00	0.06	0.09
S20094_2188	A	C	0.12	0.11	-0.13	1.00	0.94	0.88
S20094_2197	A	C	0.17	0.14	-0.17	0.00	0.02	0.19
S201_49613	T	C	0.05	0.12	0.37	0.00	0.96	1.00
S2010_26619	A	G	0.15	0.15	-0.05	0.00	0.15	0.09
S2018_27776	G	C	0.16	0.15	-0.07	0.02	0.13	0.06
S202_52326	A	T	0.04	0.10	0.38	0.93	0.00	0.00
S20278_694	T	C	0.08	0.09	0.00	0.45	0.04	0.00
S20278_697	T	C	0.16	0.16	-0.01	0.00	0.06	0.07
S203_48267	A	T	0.01	0.07	0.69	0.00	1.00	1.00
S20316_314	A	G	0.31	0.31	-0.03	0.00	0.19	0.41
S2035_4982	T	C	0.18	0.19	-0.04	0.05	0.12	0.03
S2035_5012	G	C	0.29	0.30	-0.04	0.83	0.90	0.81
S20356_2270	A	G	0.19	0.18	-0.07	0.80	0.85	0.94
S2037_24535	T	G	0.15	0.15	-0.03	0.02	0.13	0.09
S20397_183	T	C	0.13	0.17	0.08	0.59	0.04	0.06
S20404_405	A	G	0.10	0.10	-0.04	0.07	0.04	0.03
S205_22142	A	G	0.35	0.38	0.02	0.17	0.81	0.72
S20524_449	T	G	0.30	0.24	-0.25	0.25	0.27	0.16
S20557_1803	T	C	0.38	0.41	0.00	0.14	0.35	0.34
S206_37770	T	G	0.08	0.11	0.15	0.45	0.00	0.06
S2061_29036	A	G	0.28	0.33	0.08	0.18	0.25	0.20
S2063_10940	T	C	0.10	0.10	-0.10	0.86	0.96	0.94
S2063_10952	T	G	0.32	0.33	-0.02	0.05	0.17	0.16
S2066_4791	A	G	0.10	0.10	-0.07	0.02	0.06	0.03
S2066_4830	A	G	0.20	0.20	-0.06	0.98	0.92	0.88
S2067_17692	A	G	0.21	0.19	-0.14	0.91	0.88	0.94
S2067_17696	A	C	0.32	0.26	-0.21	0.07	0.12	0.28
S2067_17719	T	C	0.78	0.49	-0.67	0.80	0.56	0.59
S2073_11066	T	C	0.22	0.21	-0.08	0.02	0.12	0.16
S2079_29725	A	T	0.04	0.10	0.37	0.00	0.96	1.00
S2079_29742	A	G	0.08	0.10	0.09	1.00	0.87	0.78
S2080_13433	T	G	0.25	0.29	0.09	0.00	0.19	0.13
S20891_337	G	C	0.19	0.22	0.04	0.89	0.85	0.88
S209_76519	T	C	0.14	0.15	0.00	1.00	0.88	0.94
S2093_9942	A	C	0.23	0.18	-0.22	0.82	0.96	0.78
S2093_9962	A	G	0.52	0.49	-0.14	0.64	0.40	0.47
S2097_2052	T	C	0.43	0.49	0.06	0.73	0.42	0.53
S2098_2682	A	C	0.36	0.42	0.10	0.64	0.21	0.50
S21_4044	A	G	0.18	0.29	0.29	0.00	0.06	0.25
S21_82983	T	C	0.24	0.25	0.00	0.43	0.12	0.09
S21_83445	G	C	0.28	0.21	-0.27	0.11	0.04	0.34
S2105_1901	A	G	0.22	0.22	-0.06	0.82	0.96	0.91
S2105_1944	T	G	0.20	0.16	-0.30	0.95	1.00	0.75
S211_55359	A	G	0.15	0.14	-0.11	0.00	0.13	0.03
S211_82909	A	T	0.08	0.10	0.18	0.18	0.02	0.03
S2116_11477	T	C	0.12	0.13	0.02	0.05	0.06	0.09
S21197_1348	T	C	0.20	0.19	-0.12	0.05	0.15	0.13
S21197_1397	T	G	0.12	0.12	-0.03	0.98	0.90	0.94
S212_72892	T	G	0.12	0.10	-0.18	1.00	0.83	1.00
S212_72894	A	G	0.34	0.39	0.10	0.00	0.65	0.63
S2123_17030	T	C	0.17	0.21	0.11	0.64	0.08	0.06
S21260_1838	T	G	0.07	0.08	0.06	0.36	0.00	0.00
S21331_999	A	C	0.23	0.16	-0.44	0.93	1.00	0.75
S2149_16545	G	C	0.18	0.19	-0.03	1.00	0.79	0.84
S2149_16555	T	G	0.12	0.11	-0.21	0.98	1.00	0.91
S2149_16566	A	G	0.08	0.09	0.10	0.39	0.00	0.00
S215_33721	T	C	0.23	0.25	0.01	0.00	0.21	0.25
S21571_2222	T	C	0.10	0.11	0.02	0.25	0.00	0.03
S21579_645	A	G	0.11	0.10	-0.09	1.00	0.85	0.97
S21613_1466	T	C	0.30	0.30	-0.06	0.14	0.25	0.19
S21613_1552	G	C	0.09	0.13	0.08	0.05	0.98	1.00
S21628_1296	A	G	0.12	0.13	0.04	1.00	0.92	0.84

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S2166_26612	A	G	0.08	0.09	0.08	0.27	0.00	0.03
S2166_26627	A	G	0.09	0.11	0.07	0.70	0.00	0.00
S21671_540	A	G	0.22	0.26	0.04	0.00	0.90	0.84
S217_28536	G	C	0.14	0.15	-0.07	0.07	0.06	0.13
S217_5446	A	G	0.21	0.20	-0.11	0.16	0.13	0.16
S217_5455	T	C	0.19	0.18	-0.11	0.93	0.88	0.94
S217_68948	A	C	0.28	0.32	0.04	0.02	0.75	0.09
S21737_1490	A	C	0.19	0.20	-0.05	0.91	0.88	0.94
S21750_1189	G	C	0.10	0.10	-0.04	0.98	0.92	0.94
S21750_1288	G	C	0.20	0.20	-0.09	0.00	0.10	0.13
S218_61547	T	C	0.05	0.11	0.39	1.00	0.02	0.10
S2186_1637	T	C	0.08	0.12	0.18	0.02	0.02	0.13
S2186_22890	A	G	0.09	0.13	0.17	0.81	0.00	0.03
S21872_1572	A	G	0.12	0.12	-0.07	0.02	0.15	0.13
S21872_1581	G	C	0.25	0.18	-0.34	0.07	0.04	0.31
S21872_35	T	G	0.14	0.12	-0.14	0.91	0.83	0.94
S2189_11603	G	C	0.07	0.15	0.33	0.88	0.04	0.03
S21892_562	G	C	0.07	0.08	0.07	0.73	1.00	1.00
S21949_1240	T	C	0.09	0.10	0.01	0.82	0.98	0.97
S220_8429	T	C	0.17	0.13	-0.31	0.02	0.00	0.25
S2202_16458	T	C	0.27	0.27	-0.05	0.09	0.17	0.16
S2202_4710	A	G	0.23	0.24	-0.03	0.66	0.19	0.19
S22029_1645	T	C	0.30	0.25	-0.20	0.05	0.19	0.19
S22029_1654	A	G	0.45	0.35	-0.33	0.02	0.31	0.25
S22083_40	A	G	0.19	0.19	-0.04	0.05	0.13	0.09
S22083_48	T	C	0.16	0.17	0.02	0.02	0.12	0.03
S22247_892	A	G	0.19	0.20	-0.03	0.98	0.90	0.88
S2227_15844	T	C	0.43	0.45	-0.03	0.18	0.71	0.75
S223_67959	A	T	0.01	0.07	0.78	0.00	1.00	1.00
S22342_1893	T	C	0.10	0.10	-0.06	0.02	0.12	0.03
S22342_1926	A	C	0.29	0.32	0.04	0.68	0.88	0.81
S224_58616	A	G	0.01	0.07	0.66	0.00	1.00	1.00
S2240_6508	T	C	0.16	0.15	-0.10	0.02	0.13	0.03
S2244_19210	A	G	0.04	0.10	0.36	0.00	1.00	1.00
S22441_1660	T	C	0.10	0.10	-0.01	0.00	0.04	0.16
S22444_585	G	C	0.44	0.45	-0.03	0.20	0.38	0.47
S22444_597	A	C	0.47	0.47	-0.05	0.25	0.65	0.72
S22460_2031	G	C	0.10	0.11	0.03	1.00	0.90	0.91
S22460_2043	T	G	0.01	0.08	0.71	0.00	1.00	1.00
S225_53103	A	G	0.09	0.07	-0.23	0.27	0.02	0.00
S2251_27932	A	G	0.35	0.38	0.04	0.43	0.79	0.75
S2252_13326	T	C	0.01	0.07	0.78	0.00	1.00	1.00
S22536_2361	T	C	0.14	0.22	0.25	0.26	0.10	0.03
S2256_13845	A	C	0.03	0.10	0.62	0.00	0.98	1.00
S22588_1966	T	G	0.26	0.35	0.21	0.16	0.83	0.84
S226_48087	A	T	0.18	0.26	0.20	0.00	0.79	0.73
S226_66573	T	C	0.16	0.16	-0.05	0.00	0.21	0.06
S226_66580	G	C	0.26	0.28	0.00	0.00	0.21	0.22
S2264_14563	T	G	0.19	0.22	0.07	0.00	0.42	0.03
S22654_145	T	C	0.20	0.21	-0.04	0.95	0.88	0.78
S22654_177	T	C	0.09	0.09	-0.02	0.93	0.94	1.00
S2269_18512	G	C	0.12	0.10	-0.20	0.02	0.00	0.06
S2269_18524	T	C	0.20	0.20	-0.09	0.91	0.90	1.00
S2269_18565	A	G	0.09	0.10	0.02	0.59	0.96	0.97
S2272_19946	G	C	0.19	0.20	-0.01	0.00	0.13	0.13
S2275_27090	A	C	0.07	0.10	0.15	0.00	0.15	0.00
S2275_27109	A	G	0.01	0.08	0.82	1.00	0.00	0.00
S2278_18996	T	C	0.08	0.10	0.12	0.73	0.00	0.03
S2278_19014	T	C	0.17	0.17	-0.07	0.00	0.12	0.06
S228_12360	A	T	0.41	0.46	0.04	0.55	0.38	0.41
S2280_6381	G	C	0.14	0.12	-0.13	0.00	0.15	0.00
S2280_6407	A	T	0.18	0.16	-0.17	1.00	0.96	0.88
S2280_7440	T	G	0.72	0.47	-0.60	0.75	0.60	0.59
S22862_894	A	G	0.13	0.13	-0.07	0.07	0.10	0.03
S22862_900	A	T	0.10	0.10	-0.06	0.82	0.98	0.91
S22891_423	A	C	0.08	0.09	0.05	0.25	0.00	0.00
S22891_438	T	C	0.07	0.08	0.03	0.70	0.98	0.97
S22936_1389	T	C	0.45	0.44	-0.06	0.11	0.46	0.31
S22940_946	G	C	0.60	0.43	-0.44	0.26	0.33	0.28

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S22940_956	T	C	0.94	0.52	-0.90	0.57	0.56	0.53
S22940_960	G	C	0.14	0.11	-0.21	0.32	0.00	0.00
S22940_978	T	C	0.18	0.16	-0.12	0.07	0.02	0.06
S22940_981	A	C	0.30	0.26	-0.21	0.05	0.20	0.17
S22940_989	A	G	0.28	0.24	-0.17	0.95	0.85	0.84
S22973_51	A	G	0.15	0.14	-0.11	1.00	0.96	0.94
S22973_85	A	C	0.12	0.12	-0.10	0.14	0.08	0.03
S22973_91	T	G	0.17	0.16	-0.11	0.98	0.90	0.94
S22994_1598	A	G	0.03	0.10	0.49	1.00	0.00	0.00
S2301_27431	A	G	0.11	0.12	0.03	0.89	0.98	0.94
S2302_25434	A	G	0.11	0.12	0.00	0.00	0.06	0.03
S23029_1123	A	T	0.07	0.11	0.33	0.80	0.00	0.00
S23040_677	T	C	0.34	0.34	-0.08	0.07	0.33	0.22
S231_2491	A	C	0.08	0.07	-0.08	0.73	1.00	1.00
S2313_23695	T	C	0.18	0.20	0.04	0.00	0.13	0.06
S2313_23698	A	T	0.36	0.41	0.07	0.91	0.77	0.63
S23154_1925	A	C	0.09	0.10	-0.01	0.32	0.00	0.03
S2317_15388	T	G	0.10	0.12	0.03	0.00	0.04	0.09
S2317_15391	G	C	0.12	0.13	0.03	0.48	0.00	0.00
S2317_15392	T	C	0.13	0.13	-0.02	0.45	0.04	0.03
S232_24654	A	G	0.13	0.13	-0.03	0.07	0.08	0.06
S23226_2015	T	C	0.16	0.17	0.02	0.29	0.04	0.09
S23226_2039	G	C	0.33	0.33	-0.07	0.68	0.88	0.75
S23226_2060	T	C	0.38	0.37	-0.08	0.27	0.21	0.22
S2326_19379	A	G	0.01	0.08	0.84	0.00	1.00	1.00
S23351_2083	G	C	0.04	0.08	0.31	0.52	1.00	0.97
S2337_27776	T	C	0.36	0.37	-0.04	0.95	0.62	0.75
S2339_11991	A	G	0.17	0.15	-0.12	0.18	0.04	0.06
S2339_11998	A	G	0.26	0.23	-0.16	0.91	0.88	0.94
S2339_12011	G	C	0.99	0.53	-0.99	0.50	0.50	0.50
S23404_1527	A	C	0.40	0.41	-0.03	0.02	0.58	0.69
S2343_16866	T	C	0.13	0.11	-0.16	0.89	0.94	1.00
S23443_1674	T	G	0.99	0.53	-0.98	0.48	0.50	0.50
S23443_1678	A	G	0.15	0.14	-0.12	0.00	0.13	0.06
S23443_1704	G	C	0.34	0.28	-0.22	0.98	0.83	0.78
S23443_1715	T	G	0.15	0.14	-0.12	0.02	0.02	0.09
S23443_1721	T	C	0.67	0.43	-0.59	1.00	0.60	0.59
S23449_141	A	T	0.11	0.13	-0.01	1.00	0.88	0.94
S23449_152	T	C	0.62	0.40	-0.52	0.09	0.50	0.72
S23449_183	A	G	0.14	0.12	-0.14	0.00	0.17	0.06
S23460_1420	A	G	0.24	0.27	0.04	0.02	0.15	0.19
S235_41267	A	G	0.15	0.28	0.42	1.00	0.17	0.09
S235_66918	T	G	0.12	0.13	0.02	0.00	0.08	0.16
S2350_16067	T	G	0.42	0.28	-0.42	0.00	0.10	0.38
S23503_1996	A	C	0.10	0.13	0.14	0.11	0.06	0.03
S23549_1938	T	C	0.11	0.13	0.05	0.00	0.08	0.03
S23549_1943	T	G	0.19	0.23	0.12	0.05	0.06	0.19
S236_68122	A	G	0.10	0.11	0.10	0.52	1.00	1.00
S2361_11787	A	G	0.26	0.28	-0.01	0.64	0.12	0.16
S2363_9046	T	G	0.12	0.10	-0.13	0.95	0.90	0.97
S2363_9632	A	G	0.04	0.08	0.39	0.86	0.00	0.00
S23670_1542	A	G	0.11	0.14	0.09	0.50	1.00	0.91
S2369_6112	T	C	0.01	0.08	0.72	0.98	0.00	0.00
S237_62097	T	C	0.14	0.17	0.08	0.00	0.15	0.16
S23714_166	A	G	0.17	0.16	-0.13	0.80	0.90	0.97
S23714_179	T	C	0.30	0.26	-0.18	0.09	0.17	0.19
S2372_17509	A	T	0.14	0.16	0.03	0.91	0.90	0.91
S2372_17536	A	C	0.06	0.11	0.38	0.36	0.98	0.97
S23725_1679	T	C	0.03	0.08	0.48	1.00	0.00	0.00
S23782_569	A	G	0.22	0.23	-0.01	0.07	0.25	0.13
S23782_584	T	C	0.16	0.17	-0.01	0.00	0.06	0.07
S23782_588	G	C	0.32	0.23	-0.47	0.95	1.00	0.75
S2384_1203	A	G	0.13	0.13	-0.07	0.00	0.02	0.00
S2384_1237	T	C	0.38	0.44	0.11	0.00	0.63	0.81
S2392_9378	T	C	0.09	0.15	0.21	0.00	0.94	0.94
S2393_27336	A	C	0.11	0.11	-0.02	1.00	0.90	0.97
S23936_1965	A	C	0.09	0.14	0.22	0.00	1.00	0.97
S23943_1784	T	C	0.08	0.10	0.13	0.70	0.00	0.00
S23953_333	T	G	0.22	0.24	0.05	0.93	0.88	0.81

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S2401_8505	T	C	0.11	0.13	0.11	0.77	0.00	0.03
S24056_940	T	C	0.03	0.09	0.44	0.05	0.98	0.97
S24069_1972	A	G	0.07	0.10	0.17	0.05	0.04	0.03
S24144_626	A	G	0.41	0.33	-0.29	0.11	0.21	0.31
S24144_628	T	C	0.12	0.10	-0.13	0.00	0.04	0.27
S24144_649	A	G	0.82	0.50	-0.71	0.50	0.38	0.34
S24144_650	T	C	0.10	0.10	-0.10	0.05	0.02	0.03
S24144_656	A	G	0.96	0.52	-0.93	0.48	0.50	0.50
S24144_666	A	C	0.21	0.16	-0.29	0.93	0.98	0.85
S2419_21183	A	C	0.04	0.07	0.52	0.84	1.00	1.00
S24196_905	A	C	0.13	0.13	-0.07	0.09	0.08	0.03
S242_75929	T	C	0.01	0.07	0.64	0.00	1.00	1.00
S24206_363	A	G	0.10	0.12	0.04	0.00	0.04	0.06
S24206_387	G	C	0.21	0.22	0.01	0.66	0.87	0.81
S2421_2207	T	G	0.10	0.11	0.00	0.57	0.98	0.94
S24222_966	T	C	0.39	0.48	0.13	0.41	0.48	0.47
S24222_989	A	T	0.18	0.22	0.17	0.64	0.92	0.91
S2423_14971	T	C	0.43	0.44	-0.03	0.02	0.54	0.50
S24242_618	G	C	0.10	0.12	0.10	0.00	0.06	0.06
S2429_7011	T	C	0.11	0.13	0.06	1.00	0.94	0.88
S2429_7013	T	C	0.04	0.11	0.42	0.00	0.94	1.00
S2435_6458	A	G	0.18	0.19	0.01	0.64	1.00	0.97
S2436_9163	A	G	0.12	0.11	-0.08	0.00	0.08	0.06
S2436_9171	A	G	0.23	0.24	-0.05	0.09	0.13	0.03
S2437_13223	A	G	0.33	0.33	-0.04	0.91	0.75	0.75
S24370_1625	A	T	0.09	0.12	0.10	0.34	1.00	0.97
S24370_1631	A	G	0.08	0.12	0.17	0.00	0.06	0.00
S2440_5725	A	T	0.07	0.14	0.36	0.00	1.00	1.00
S24480_1361	G	C	0.42	0.48	0.08	0.32	0.50	0.53
S245_43973	A	T	0.27	0.35	0.18	1.00	0.20	0.06
S245_83200	T	C	0.12	0.13	-0.05	0.02	0.15	0.00
S245_83206	T	G	0.10	0.10	-0.04	0.00	0.02	0.03
S245_86399	A	G	0.14	0.15	0.05	0.00	0.12	0.06
S24509_1889	T	C	0.11	0.13	0.08	0.57	0.04	0.00
S24525_916	T	G	0.44	0.27	-0.66	0.64	0.63	0.97
S24582_1588	T	C	0.21	0.22	-0.02	0.23	0.17	0.09
S24584_1450	T	G	0.06	0.11	0.32	0.57	0.98	0.94
S24760_668	T	C	0.42	0.44	0.02	0.02	0.54	0.31
S2479_4353	T	C	0.07	0.08	0.21	0.57	1.00	1.00
S2481_8003	T	C	0.09	0.09	-0.05	0.00	0.08	0.06
S2482_12089	A	G	0.09	0.10	0.01	0.50	0.00	0.00
S2482_12096	A	G	0.11	0.12	0.00	1.00	0.94	0.94
S24839_236	T	C	0.20	0.22	-0.01	1.00	0.87	0.97
S24851_1855	T	C	0.34	0.32	-0.07	0.00	0.87	0.78
S24857_1893	T	C	0.11	0.13	0.04	0.11	0.04	0.00
S24857_1945	T	G	0.12	0.12	-0.02	0.00	0.02	0.16
S24857_1947	T	G	0.25	0.21	-0.19	0.18	0.21	0.19
S24861_1072	T	C	1.00	0.53	-1.00	0.50	0.50	0.50
S24869_2064	T	C	0.08	0.13	0.25	0.80	0.94	0.97
S24886_1634	A	T	0.28	0.25	-0.16	0.43	0.19	0.03
S24886_1673	A	G	0.13	0.12	-0.09	0.14	0.04	0.06
S2493_26519	A	C	0.39	0.38	-0.07	0.00	0.37	0.28
S24945_1546	A	G	0.04	0.10	0.46	0.05	1.00	1.00
S25_142156	T	C	0.10	0.20	0.32	0.00	0.94	0.97
S25027_828	T	G	0.26	0.42	0.36	1.00	0.71	0.47
S25086_1616	A	G	0.26	0.27	-0.01	0.75	0.87	0.81
S25128_1465	T	C	0.14	0.15	0.01	0.09	0.04	0.13
S2514_24373	A	G	0.10	0.12	0.03	0.02	0.12	0.03
S2514_24392	A	G	0.16	0.16	-0.07	0.00	0.10	0.03
S2514_24418	A	C	0.13	0.13	-0.06	1.00	0.92	0.91
S2518_17665	A	G	0.07	0.08	0.05	0.50	0.02	0.00
S25183_1618	A	T	0.15	0.18	0.08	0.02	0.19	0.07
S25183_1619	A	G	0.10	0.12	0.04	0.95	0.90	0.93
S2520_6131	G	C	0.16	0.17	0.01	1.00	0.90	0.84
S2520_6157	T	C	0.11	0.12	0.03	0.00	0.12	0.03
S2523_25799	T	G	0.10	0.17	0.22	0.07	0.94	0.88
S2523_25810	A	G	0.08	0.11	0.10	0.00	0.10	0.06
S25252_1585	A	G	0.24	0.25	0.06	0.05	0.12	0.19
S25252_1604	A	C	0.14	0.13	-0.13	0.02	0.04	0.13

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S253_9826	T	C	0.21	0.27	0.14	0.00	0.19	0.31
S25324_1726	A	G	0.04	0.08	0.37	0.98	0.00	0.03
S25324_1727	A	C	0.10	0.09	-0.07	1.00	0.94	0.88
S25507_2163	A	G	0.25	0.28	0.03	0.05	0.08	0.28
S25507_2165	T	G	0.29	0.22	-0.28	0.95	0.90	0.75
S2570_21156	G	C	0.03	0.09	0.54	1.00	0.02	0.00
S25701_520	T	C	0.13	0.12	-0.11	0.02	0.06	0.19
S25701_531	A	G	0.95	0.53	-0.90	0.50	0.48	0.47
S25701_543	A	G	0.13	0.12	-0.12	0.16	0.06	0.09
S2578_11149	A	T	0.10	0.11	0.00	0.00	0.02	0.06
S25796_497	A	G	0.45	0.43	-0.11	0.32	0.33	0.25
S25796_525	T	G	0.39	0.26	-0.55	0.59	0.58	0.97
S2580_18629	A	C	0.35	0.35	-0.04	0.84	0.21	0.22
S2583_19240	A	C	0.08	0.12	0.19	0.95	0.94	0.97
S2593_3621	A	T	0.12	0.17	0.19	0.00	0.13	0.13
S2593_3654	A	G	0.07	0.09	0.22	0.36	1.00	1.00
S26_66001	T	G	0.02	0.07	0.63	1.00	0.00	0.00
S260_65863	A	C	0.12	0.17	0.19	0.43	0.06	0.06
S260_80745	A	G	0.24	0.23	-0.06	0.95	0.83	0.88
S261_56233	A	T	0.14	0.19	0.12	0.95	0.90	0.94
S2611_19314	T	C	0.10	0.11	0.01	0.50	0.00	0.06
S26140_974	A	T	0.21	0.21	-0.04	0.05	0.23	0.13
S26140_990	A	G	0.16	0.15	-0.05	0.02	0.06	0.09
S2615_10191	T	G	0.18	0.21	0.06	0.73	0.04	0.16
S26156_2030	T	C	0.11	0.12	0.03	0.02	0.04	0.06
S26156_2048	T	C	0.11	0.13	0.00	0.23	0.04	0.03
S26156_2071	A	G	0.12	0.13	0.00	0.02	0.12	0.09
S2616_12165	T	C	0.12	0.16	0.11	0.05	0.12	0.03
S26167_1608	A	C	0.15	0.15	-0.07	0.02	0.12	0.06
S262_31577	T	C	0.04	0.10	0.51	0.89	0.00	0.00
S2625_7737	A	G	0.06	0.11	0.34	0.77	0.04	0.00
S2629_6149	A	T	0.11	0.12	-0.03	0.48	1.00	1.00
S263_47478	A	G	0.08	0.11	0.18	0.68	0.00	0.00
S2632_16360	T	G	0.34	0.33	-0.08	0.95	0.81	0.72
S26328_206	A	G	0.29	0.36	0.12	0.48	0.21	0.34
S2634_4417	T	C	0.04	0.09	0.53	0.00	1.00	1.00
S2635_3016	A	G	0.40	0.44	0.05	0.00	0.46	0.25
S264_75686	T	C	0.02	0.08	0.59	0.00	1.00	1.00
S2645_841	G	C	0.23	0.26	0.07	0.00	0.88	0.81
S26657_1588	A	C	0.20	0.17	-0.18	0.93	1.00	0.84
S26709_1767	G	C	0.15	0.16	0.01	0.75	0.98	1.00
S26709_1773	G	C	0.12	0.13	-0.05	0.14	0.06	0.03
S26744_1051	T	C	0.11	0.14	0.15	0.52	1.00	0.97
S26771_1809	T	G	0.12	0.14	0.00	0.00	0.04	0.03
S268_56911	A	G	0.08	0.11	0.10	0.50	0.00	0.00
S26868_318	T	C	0.09	0.10	0.03	1.00	0.94	1.00
S26876_105	T	C	0.12	0.11	-0.06	0.09	0.04	0.03
S26876_152	A	G	0.27	0.18	-0.50	0.02	0.00	0.31
S26903_466	A	G	0.07	0.13	0.29	0.02	0.92	0.88
S26955_242	A	G	0.12	0.12	-0.10	0.98	0.88	0.91
S26955_266	G	C	0.16	0.19	0.06	0.86	0.02	0.06
S26955_281	T	C	0.20	0.19	-0.06	0.05	0.06	0.06
S2698_13170	T	C	0.23	0.17	-0.40	0.98	1.00	0.72
S2698_13172	T	C	0.10	0.11	0.06	0.55	0.00	0.00
S2698_13190	A	G	0.11	0.10	-0.10	0.02	0.06	0.09
S27_148045	T	C	0.07	0.10	0.12	1.00	0.96	1.00
S27_148056	A	G	0.08	0.14	0.31	0.70	0.00	0.00
S270_29109	A	G	0.15	0.21	0.15	0.00	0.85	0.97
S2703_17068	A	G	0.08	0.10	0.07	0.02	0.10	0.06
S27038_272	G	C	0.07	0.09	0.14	0.81	0.98	1.00
S27168_230	G	C	0.39	0.38	-0.08	0.11	0.33	0.34
S2717_17492	T	C	0.07	0.07	-0.03	0.23	0.00	0.00
S2717_19726	A	G	0.38	0.25	-0.54	0.71	0.58	0.97
S27220_1653	T	C	0.14	0.15	0.00	0.02	0.15	0.16
S27220_1686	T	G	0.25	0.19	-0.29	0.80	0.73	0.97
S27222_1278	T	G	0.06	0.10	0.27	0.80	0.00	0.00
S2723_14225	T	C	0.24	0.21	-0.18	0.02	0.13	0.22
S2723_7125	G	C	0.83	0.48	-0.78	0.93	0.50	0.53
S2723_7174	A	C	0.12	0.10	-0.31	1.00	1.00	0.94

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S2724_8067	A	T	0.31	0.30	-0.07	1.00	0.73	0.78
S2725_14072	A	G	0.40	0.39	-0.08	0.30	0.19	0.28
S2725_14105	T	G	0.46	0.45	-0.08	0.55	0.38	0.34
S27291_36	A	G	0.16	0.18	0.04	0.09	0.02	0.00
S27291_60	T	C	0.09	0.11	0.08	0.00	0.08	0.00
S27337_1902	A	C	0.15	0.14	-0.13	0.00	0.12	0.16
S27337_1903	A	T	0.15	0.12	-0.15	0.32	0.08	0.06
S27337_1907	A	G	0.20	0.17	-0.20	0.00	0.13	0.06
S27337_1931	A	T	0.14	0.13	-0.11	1.00	0.87	0.97
S27337_1936	A	G	0.37	0.30	-0.25	0.20	0.25	0.25
S27337_1955	A	G	0.40	0.32	-0.27	0.80	0.79	0.75
S27387_1748	A	G	0.02	0.09	0.71	0.32	1.00	1.00
S2746_17836	T	C	0.11	0.10	-0.11	0.00	0.02	0.22
S2746_20612	A	C	0.14	0.14	-0.09	0.00	0.12	0.03
S2746_20639	A	C	0.18	0.14	-0.21	0.95	1.00	0.81
S27499_1085	A	G	0.34	0.38	0.05	1.00	0.69	0.75
S27499_1095	T	C	0.17	0.18	0.02	0.00	0.12	0.28
S2750_2108	A	C	0.43	0.45	-0.01	0.23	0.40	0.41
S276_10492	A	C	0.31	0.30	-0.08	0.00	0.19	0.19
S277_51446	A	T	0.07	0.12	0.33	0.64	0.02	0.00
S27703_519	A	C	0.12	0.12	-0.06	0.02	0.10	0.06
S27728_1408	A	T	0.12	0.13	-0.01	1.00	0.85	0.94
S27728_1425	T	C	0.20	0.21	0.00	0.00	0.08	0.25
S2778_5837	G	C	0.28	0.20	-0.48	0.95	1.00	0.78
S27780_826	T	C	0.37	0.38	-0.03	0.09	0.29	0.25
S27843_1331	A	G	0.13	0.11	-0.19	0.93	1.00	0.88
S2790_7128	A	C	0.13	0.14	-0.04	0.09	0.06	0.00
S27902_676	T	C	0.10	0.17	0.25	0.00	0.10	0.97
S27940_495	A	T	0.48	0.50	-0.02	0.52	0.46	0.44
S2796_17938	T	C	0.10	0.13	0.07	1.00	0.83	0.93
S2796_23807	A	G	0.02	0.08	0.65	0.00	1.00	1.00
S27990_202	A	G	0.09	0.13	0.16	0.00	0.06	0.10
S28_122084	T	C	0.25	0.29	0.05	0.00	0.83	0.78
S28_88523	T	G	0.16	0.16	-0.04	0.00	0.10	0.09
S2802_1172	T	C	0.11	0.16	0.11	0.95	0.10	0.03
S281_34109	A	T	0.03	0.12	0.64	1.00	0.00	0.00
S2814_23716	A	C	0.33	0.35	0.00	0.20	0.23	0.13
S28249_971	G	C	0.22	0.21	-0.08	0.00	0.13	0.16
S283_40625	G	C	0.17	0.13	-0.30	0.07	0.00	0.09
S283_42792	A	G	0.15	0.16	0.00	0.61	1.00	0.84
S28331_918	A	C	0.23	0.20	-0.19	0.11	0.08	0.19
S28335_197	T	C	0.43	0.45	-0.02	0.48	0.60	0.63
S28354_813	A	C	0.53	0.28	-0.89	0.57	0.50	0.97
S28354_839	A	C	0.20	0.21	0.01	0.05	0.12	0.19
S28357_793	A	C	0.15	0.14	-0.09	0.07	0.02	0.09
S28384_1511	A	G	0.09	0.11	0.04	0.02	0.08	0.03
S28423_1615	T	C	0.08	0.09	0.05	0.24	0.02	0.00
S2844_12004	T	G	0.13	0.18	0.15	0.27	0.98	0.91
S28440_632	A	T	0.43	0.46	0.02	0.27	0.33	0.47
S28573_1470	T	G	0.14	0.11	-0.28	0.00	0.00	0.16
S28573_1483	T	C	0.90	0.50	-0.87	0.91	0.50	0.50
S286_76550	A	G	0.28	0.24	-0.20	0.00	0.10	0.19
S286_76551	T	G	0.97	0.53	-0.94	0.50	0.52	0.53
S286_76554	T	C	0.98	0.52	-0.97	0.50	0.50	0.50
S286_76557	T	G	0.83	0.50	-0.74	0.23	0.48	0.44
S2861_756	A	T	0.10	0.12	0.08	0.07	0.08	0.09
S28648_226	T	C	0.45	0.46	-0.03	0.43	0.63	0.84
S287_32278	A	G	0.07	0.07	0.05	0.27	0.00	0.00
S28720_1949	A	T	0.14	0.16	0.03	0.98	0.96	0.94
S2880_2477	A	G	0.13	0.14	-0.01	0.16	0.15	0.06
S2881_12031	A	C	0.10	0.11	0.00	0.00	0.08	0.06
S2881_12033	A	G	0.11	0.12	-0.03	0.00	0.04	0.06
S28814_1278	A	C	0.36	0.44	0.13	0.86	0.48	0.19
S28814_1366	A	G	0.11	0.11	-0.04	0.64	1.00	0.97
S2885_1657	A	G	0.47	0.35	-0.35	0.32	0.15	0.28
S28885_1030	T	C	0.18	0.21	0.05	0.00	0.15	0.03
S289_35889	T	C	0.08	0.13	0.25	0.80	0.02	0.00
S2896_793	T	C	0.15	0.16	-0.03	0.14	0.12	0.03
S28965_537	A	G	0.23	0.23	-0.05	0.02	0.15	0.28

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S28965_576	A	T	0.10	0.09	-0.09	0.75	0.96	1.00
S2900_18426	A	G	0.36	0.29	-0.26	0.00	0.31	0.28
S2900_19607	A	T	0.35	0.29	-0.26	0.00	0.21	0.19
S2900_19617	A	C	0.36	0.30	-0.24	1.00	0.73	0.78
S29002_296	A	C	0.17	0.15	-0.16	0.75	0.92	1.00
S29002_297	T	C	0.17	0.14	-0.17	0.64	0.96	1.00
S29002_306	A	T	0.18	0.17	-0.09	0.00	0.10	0.06
S29006_1865	T	G	0.17	0.16	-0.11	0.02	0.13	0.09
S29006_1878	T	C	0.15	0.14	-0.12	0.00	0.17	0.06
S29006_1879	A	G	0.22	0.20	-0.16	0.00	0.04	0.16
S29054_1698	A	T	0.11	0.10	-0.09	0.95	0.90	0.97
S291_20665	A	G	0.22	0.21	-0.11	0.00	0.19	0.22
S2919_9787	A	C	0.07	0.11	0.22	0.80	0.00	0.00
S2923_9039	A	C	0.08	0.10	0.09	0.00	0.13	0.00
S29267_823	T	C	0.05	0.06	0.23	0.14	0.00	0.00
S29302_1257	T	C	0.53	0.44	-0.26	0.70	0.60	0.88
S29320_1904	T	G	0.10	0.09	-0.11	0.98	0.88	0.97
S2946_16623	T	C	0.25	0.29	0.07	0.00	0.10	0.34
S2947_20789	G	C	0.06	0.08	0.18	0.38	0.00	0.00
S29474_654	T	C	0.08	0.10	0.07	0.05	0.12	0.03
S2950_10444	A	G	0.10	0.13	0.13	0.64	0.02	0.03
S2956_7939	T	G	0.03	0.08	0.50	0.98	0.00	0.00
S2958_775	T	C	0.16	0.19	0.07	0.05	0.19	0.13
S2958_801	T	G	0.14	0.15	-0.01	0.09	0.06	0.16
S2958_815	A	G	0.09	0.11	0.08	0.98	0.94	0.97
S29590_820	T	C	0.37	0.40	0.02	0.18	0.71	0.73
S296_20919	G	C	0.41	0.33	-0.27	0.64	0.90	0.75
S29661_1324	A	G	0.47	0.35	-0.37	0.86	0.67	0.63
S29661_1354	T	C	0.15	0.14	-0.13	0.00	0.19	0.03
S29661_1357	G	C	0.31	0.26	-0.21	0.98	0.67	0.78
S29661_1360	A	G	0.17	0.15	-0.14	0.23	0.06	0.03
S297_39338	A	C	0.52	0.35	-0.46	0.82	0.85	0.56
S297_44550	T	G	0.07	0.10	0.22	0.57	1.00	1.00
S29790_63	T	C	0.02	0.09	0.55	1.00	0.00	0.00
S29791_875	T	C	0.45	0.48	0.00	0.55	0.58	0.75
S298_26914	T	G	0.14	0.16	0.02	0.02	0.17	0.03
S298_34517	A	G	0.11	0.11	-0.05	0.02	0.04	0.16
S29840_1696	T	C	0.18	0.17	-0.09	0.43	0.06	0.06
S3_11284	A	G	0.22	0.22	-0.04	0.00	0.17	0.19
S3_378016	T	C	0.14	0.16	0.02	0.00	0.13	0.13
S3_378038	A	G	0.06	0.12	0.32	0.50	0.00	0.07
S3_83237	A	C	0.09	0.11	0.04	0.00	0.08	0.00
S300_26370	T	C	0.18	0.14	-0.32	0.07	0.00	0.13
S300_26415	T	C	0.01	0.07	0.75	1.00	0.00	0.00
S301_26101	A	G	0.37	0.36	-0.10	0.59	0.69	0.91
S30144_1055	T	G	0.05	0.11	0.35	0.14	0.98	0.97
S30144_1168	T	G	0.15	0.15	-0.05	0.00	0.04	0.09
S30186_1916	T	C	0.17	0.16	-0.09	0.16	0.06	0.06
S30245_1716	G	C	0.46	0.50	0.04	0.57	0.42	0.50
S3026_12283	A	G	0.04	0.10	0.54	0.00	1.00	1.00
S303_48932	T	G	0.02	0.08	0.61	0.00	1.00	1.00
S3031_7442	A	T	0.19	0.18	-0.10	0.00	0.12	0.13
S30321_981	A	G	0.15	0.16	-0.03	0.34	0.06	0.09
S3034_16448	A	C	0.27	0.19	-0.46	0.90	1.00	0.70
S3034_16552	A	C	0.10	0.13	0.13	0.02	0.08	0.10
S3035_14704	A	G	0.08	0.08	0.02	0.23	0.00	0.06
S3043_10736	T	C	0.10	0.12	0.07	0.34	0.00	0.09
S3046_15808	G	C	0.08	0.11	0.15	1.00	0.94	0.91
S3046_15863	G	C	0.08	0.10	0.07	0.98	0.96	0.94
S30479_1485	A	G	0.11	0.11	-0.05	0.89	0.98	0.94
S3059_4218	A	T	0.16	0.14	-0.12	0.98	0.85	0.94
S3059_4238	G	C	0.21	0.16	-0.23	0.59	0.83	0.97
S3061_15276	A	T	0.22	0.28	0.12	0.09	0.23	0.28
S3077_9381	A	G	0.20	0.29	0.21	0.86	0.06	0.19
S3077_9445	T	C	0.29	0.35	0.11	0.18	0.33	0.16
S308_58820	A	G	0.20	0.23	0.06	0.05	0.21	0.31
S308_58821	T	C	0.15	0.17	0.04	0.59	0.96	0.97
S3081_10728	T	C	0.30	0.35	0.05	0.55	0.79	0.88
S3087_3820	T	G	0.18	0.15	-0.20	0.07	0.02	0.31

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S3088_7528	A	G	0.49	0.47	-0.10	0.23	0.63	0.47
S309_31041	A	C	0.45	0.44	-0.06	0.02	0.44	0.41
S31_105537	G	C	0.09	0.10	0.07	1.00	0.83	1.00
S31_30220	A	C	0.97	0.53	-0.95	0.43	0.50	0.50
S31_38736	A	T	0.09	0.11	0.05	0.00	0.10	0.00
S31_64036	A	T	0.09	0.12	0.17	0.45	1.00	1.00
S31_68299	T	C	0.13	0.13	-0.04	0.00	0.06	0.06
S3108_22686	A	G	0.07	0.10	0.16	0.98	0.85	0.97
S311_22288	A	T	0.23	0.20	-0.17	1.00	0.81	0.75
S311_27336	A	T	0.16	0.20	0.07	1.00	0.77	0.91
S3110_17107	T	T	0.10	0.11	-0.01	1.00	0.90	1.00
S3110_17110	A	G	0.13	0.13	-0.07	0.11	0.02	0.13
S3110_17122	G	C	0.17	0.16	-0.10	1.00	0.92	0.84
S3118_15787	A	G	0.19	0.18	-0.10	0.02	0.15	0.03
S3120_4739	A	T	0.08	0.10	0.18	0.34	0.00	0.00
S313_32620	T	C	0.19	0.19	-0.04	0.59	1.00	0.94
S3134_16869	T	C	0.15	0.14	-0.12	0.14	0.06	0.06
S3134_16890	T	G	0.14	0.13	-0.10	0.05	0.04	0.07
S3136_6623	A	G	0.45	0.47	-0.02	0.43	0.71	0.63
S3139_20881	A	C	0.28	0.29	-0.04	0.00	0.25	0.13
S3139_20893	T	C	0.15	0.15	-0.05	0.05	0.10	0.09
S3139_5470	T	G	0.07	0.07	0.03	0.18	0.00	0.00
S3157_2440	T	G	0.23	0.30	0.15	1.00	0.23	0.16
S316_63381	T	C	0.10	0.16	0.17	0.89	0.06	0.03
S3167_5715	A	G	0.01	0.08	0.71	1.00	0.00	0.00
S318_47664	A	G	0.02	0.08	0.71	0.45	0.00	0.00
S318_47686	A	G	0.03	0.08	0.55	0.55	0.00	0.00
S319_37353	T	C	0.17	0.18	-0.02	0.00	0.12	0.16
S319_37374	A	G	0.09	0.12	0.07	0.98	0.96	0.91
S319_52687	G	C	0.17	0.14	-0.25	0.09	0.00	0.25
S319_57362	G	C	0.15	0.15	-0.07	1.00	0.87	0.94
S319_57413	T	G	0.11	0.11	0.00	0.05	0.04	0.16
S319_77693	A	G	0.18	0.16	-0.17	0.86	0.96	0.84
S3196_15557	T	C	0.19	0.19	-0.08	0.00	0.12	0.06
S3199_5708	G	C	0.06	0.11	0.35	0.73	0.00	0.00
S320_73650	A	G	0.42	0.43	-0.02	0.02	0.65	0.69
S3203_9892	A	G	0.10	0.13	0.15	0.05	0.13	0.00
S3203_9934	T	C	0.15	0.21	0.21	0.95	0.79	1.00
S3204_21245	A	G	0.13	0.13	-0.07	0.98	0.88	0.94
S3205_12808	A	G	0.15	0.16	0.00	0.59	0.02	0.03
S3207_13302	A	T	0.10	0.11	-0.04	0.95	0.94	0.93
S3207_13318	G	C	0.12	0.14	0.01	0.70	0.96	0.93
S3210_1994	T	G	0.15	0.15	-0.08	0.91	0.88	0.88
S3210_2018	A	G	0.28	0.28	-0.08	0.32	0.12	0.13
S322_48000	A	G	0.01	0.07	0.78	1.00	0.00	0.00
S3239_21105	A	G	0.11	0.13	0.01	0.00	0.10	0.03
S3240_18391	A	G	0.14	0.11	-0.26	0.98	1.00	0.91
S3240_18398	T	G	0.31	0.31	-0.03	0.05	0.25	0.16
S3240_18428	T	C	0.15	0.16	-0.05	0.93	0.90	0.84
S3240_18431	T	C	0.17	0.16	-0.05	0.00	0.12	0.09
S3286_21139	T	C	0.10	0.10	-0.07	0.02	0.02	0.00
S3295_7265	T	C	0.08	0.09	0.13	0.32	0.02	0.00
S331_58168	T	C	0.14	0.16	0.04	1.00	0.83	0.97
S331_6966	A	G	0.14	0.15	-0.02	0.02	0.13	0.03
S331_6972	T	C	0.11	0.11	-0.05	0.00	0.00	0.03
S331_6987	A	T	0.05	0.09	0.34	0.86	0.00	0.00
S3310_7685	G	C	0.22	0.30	0.21	1.00	0.85	0.22
S333_3501	A	T	0.09	0.11	0.06	1.00	0.96	0.94
S333_3512	A	G	0.12	0.14	0.00	0.02	0.10	0.06
S3333_7903	A	G	0.08	0.11	0.11	0.02	0.02	0.22
S3333_922	G	C	0.25	0.21	-0.20	0.09	0.06	0.28
S3335_347	T	C	0.27	0.28	-0.06	0.00	0.19	0.09
S334_1132	A	G	0.06	0.09	0.30	0.74	1.00	0.93
S3341_13068	A	G	0.13	0.15	0.01	0.05	0.16	0.03
S3348_16469	A	C	0.16	0.17	-0.02	0.14	0.10	0.06
S3348_1791	A	G	0.32	0.33	-0.04	0.32	0.81	0.81
S335_74064	T	C	0.01	0.09	0.84	0.81	0.00	0.00
S3355_501	A	G	0.34	0.44	0.15	0.32	0.42	0.47
S3370_19885	A	G	0.09	0.11	0.10	0.23	1.00	1.00

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S3380_7605	T	C	0.13	0.13	-0.04	0.00	0.10	0.06
S34_12552	T	C	0.14	0.14	-0.07	1.00	0.88	0.88
S3401_6281	G	C	0.22	0.23	-0.01	0.00	0.23	0.16
S3405_2909	T	G	0.11	0.11	-0.04	0.02	0.10	0.09
S3405_2957	A	G	0.12	0.17	0.19	0.14	0.08	0.03
S3429_11906	A	C	0.06	0.09	0.20	0.05	0.04	0.00
S3429_8996	A	T	0.34	0.40	0.10	0.16	0.40	0.25
S3435_13967	T	G	0.62	0.43	-0.48	0.61	0.58	0.78
S3435_13993	T	C	0.12	0.11	-0.09	0.02	0.08	0.09
S3435_14005	G	C	0.25	0.24	-0.10	0.98	0.83	0.84
S3437_6969	A	G	0.21	0.26	0.14	0.93	0.90	0.88
S3437_7003	G	C	0.05	0.11	0.40	0.84	0.00	0.03
S344_18410	G	C	0.20	0.22	0.02	0.95	0.92	0.91
S344_24824	T	C	0.18	0.18	-0.02	0.05	0.10	0.13
S344_24833	T	C	0.15	0.16	-0.02	0.02	0.17	0.06
S344_24841	A	G	0.45	0.48	0.00	0.75	0.52	0.44
S344_5834	A	G	0.07	0.10	0.19	0.95	0.96	0.93
S3451_13888	T	C	0.06	0.13	0.32	0.02	0.92	1.00
S347_57293	T	G	0.42	0.32	-0.30	0.41	0.15	0.16
S347_57308	A	G	0.49	0.36	-0.37	0.02	0.38	0.31
S347_57318	T	C	0.29	0.26	-0.14	0.00	0.08	0.22
S347_57334	T	G	0.49	0.38	-0.34	0.36	0.23	0.25
S3473_10409	A	C	0.52	0.49	-0.12	0.23	0.46	0.50
S3473_15453	A	C	0.32	0.34	0.00	0.05	0.37	0.22
S3473_15467	A	G	0.13	0.13	-0.03	0.00	0.00	0.16
S348_31301	A	C	0.09	0.09	-0.02	0.98	0.96	1.00
S3481_15442	A	G	0.13	0.12	-0.10	0.98	0.94	0.94
S3482_617	T	C	0.12	0.12	-0.09	0.00	0.08	0.06
S3482_636	T	C	0.34	0.28	-0.22	0.07	0.23	0.16
S3482_652	A	G	0.22	0.19	-0.18	0.05	0.13	0.03
S3483_4203	T	G	0.09	0.12	0.14	0.00	0.08	0.03
S3487_9858	A	G	0.07	0.10	0.13	0.00	0.06	0.00
S3487_9867	T	C	0.20	0.23	0.07	0.59	0.88	0.91
S3490_9993	A	C	0.47	0.49	-0.03	0.27	0.48	0.53
S3493_14919	G	C	0.34	0.42	0.15	1.00	0.58	0.50
S35_100657	A	G	0.15	0.13	-0.16	0.93	1.00	0.91
S35_27129	T	C	0.09	0.11	0.16	0.80	0.00	0.00
S35_61530	A	C	0.17	0.16	-0.08	0.00	0.12	0.09
S351_41429	A	G	0.02	0.08	0.66	0.02	0.96	1.00
S351_41809	T	G	0.12	0.17	0.17	0.00	0.06	0.16
S3513_1258	A	C	0.05	0.11	0.31	1.00	0.04	0.03
S3531_13110	T	C	0.26	0.27	-0.01	0.80	0.04	0.38
S3541_8542	T	C	0.11	0.11	-0.05	0.05	0.12	0.16
S3545_20160	T	C	0.13	0.13	-0.06	0.02	0.08	0.03
S3545_20166	A	C	0.11	0.12	0.02	1.00	0.90	0.94
S3548_16668	T	C	0.24	0.25	-0.03	0.00	0.85	0.19
S3556_16064	G	C	0.08	0.11	0.11	0.29	0.02	0.06
S356_41387	T	C	0.22	0.23	0.00	0.50	0.90	0.88
S3567_9958	G	C	0.37	0.37	-0.05	0.82	0.81	0.63
S357_34790	A	C	0.04	0.11	0.40	1.00	0.00	0.06
S359_52989	T	C	0.54	0.42	-0.34	0.75	0.35	0.13
S359_53102	A	G	0.11	0.11	-0.10	1.00	0.94	0.97
S359_53135	A	G	0.21	0.19	-0.12	1.00	0.96	0.94
S36_122047	A	G	0.11	0.10	-0.02	0.50	0.00	0.06
S36_128331	A	G	0.12	0.15	0.15	0.05	0.08	0.06
S36_128335	T	C	0.18	0.24	0.15	0.02	0.19	0.13
S36_128339	A	T	0.12	0.17	0.16	0.00	0.04	0.28
S3603_7830	A	G	0.14	0.13	-0.11	0.00	0.13	0.06
S3606_3467	A	G	0.14	0.14	-0.06	0.00	0.08	0.13
S3612_9416	G	G	0.08	0.09	0.02	1.00	0.08	1.00
S3629_11688	T	C	0.20	0.16	-0.19	0.55	1.00	0.97
S3634_1296	T	C	0.26	0.27	-0.02	0.00	0.23	0.34
S365_5873	A	G	0.10	0.10	-0.11	0.93	0.94	1.00
S3663_13496	T	C	0.09	0.11	0.04	0.97	0.96	1.00
S3663_7953	A	G	0.21	0.21	-0.04	0.98	0.83	0.88
S3689_732	A	C	0.45	0.26	-0.74	0.93	1.00	0.56
S3689_736	T	C	0.14	0.13	-0.11	0.09	0.10	0.13
S3689_749	A	C	0.97	0.53	-0.95	0.50	0.50	0.53
S3693_12263	A	G	0.42	0.46	0.05	0.18	0.76	0.40

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S3693_12312	A	G	0.12	0.17	0.20	0.07	0.11	0.13
S37_38798	A	G	0.12	0.19	0.27	0.00	0.35	0.09
S37_38808	T	C	0.11	0.16	0.18	0.00	0.08	0.06
S3703_6025	T	C	0.40	0.46	0.08	0.75	0.40	0.19
S3704_38	A	G	0.11	0.12	0.03	0.02	0.06	0.03
S3704_68	T	C	0.12	0.12	-0.06	0.02	0.04	0.03
S3704_84	T	C	0.09	0.11	0.06	0.00	0.06	0.09
S3723_15240	A	T	0.17	0.22	0.13	1.00	0.88	0.78
S3729_5218	A	C	0.15	0.17	0.01	0.41	0.04	0.16
S3735_8242	A	T	0.12	0.12	-0.04	1.00	0.94	0.91
S375_50343	A	G	0.30	0.32	0.01	0.00	0.06	0.59
S3758_13711	T	C	0.23	0.22	-0.07	0.11	0.06	0.19
S3770_11549	A	G	0.46	0.46	-0.07	0.82	0.63	0.63
S3770_11570	T	C	0.28	0.28	-0.05	0.02	0.29	0.16
S3770_14245	A	C	0.24	0.19	-0.28	0.70	0.65	0.97
S378_64411	A	G	0.32	0.40	0.14	1.00	0.27	0.31
S3787_14192	T	G	0.30	0.37	0.14	0.66	0.73	0.77
S3787_14218	A	G	0.33	0.40	0.10	0.43	0.83	0.63
S3794_1199	A	G	0.19	0.23	0.08	0.70	0.90	0.84
S38_40909	T	C	0.42	0.45	0.02	0.59	0.62	0.78
S38_86045	G	C	0.55	0.40	-0.42	0.98	0.65	0.66
S3800_13501	A	C	0.19	0.20	0.01	0.82	0.92	0.97
S3801_9052	A	G	0.18	0.18	-0.07	0.95	0.88	0.91
S381_51334	A	G	0.17	0.19	0.02	0.00	0.08	0.16
S381_9685	A	G	0.08	0.11	0.13	0.00	0.12	0.00
S381_9731	A	G	0.18	0.19	-0.02	0.00	0.23	0.03
S384_64556	A	C	0.22	0.22	-0.08	1.00	0.46	0.97
S3850_15776	T	C	0.41	0.42	-0.04	0.34	0.81	0.66
S3877_2093	A	C	0.35	0.28	-0.26	0.20	0.23	0.22
S389_30867	T	C	0.10	0.12	0.05	0.09	0.92	1.00
S3900_7251	A	G	0.51	0.48	-0.13	0.61	0.35	0.47
S3900_7297	T	G	0.51	0.28	-0.85	0.57	0.52	0.97
S3903_12579	T	C	0.01	0.08	0.77	1.00	0.00	0.00
S3906_10557	T	G	0.11	0.10	-0.14	1.00	0.88	0.97
S3916_8696	G	C	0.11	0.11	-0.03	0.89	0.96	0.91
S3916_8730	T	C	0.15	0.17	0.04	0.43	1.00	0.97
S3916_8735	T	G	0.11	0.12	-0.01	0.05	0.08	0.09
S392_66561	G	C	0.43	0.28	-0.49	0.12	0.04	0.34
S393_36579	A	G	0.08	0.09	0.03	0.00	0.02	0.00
S393_60056	G	C	0.04	0.07	0.29	0.36	0.00	0.00
S3933_11464	T	C	0.43	0.44	-0.05	0.43	0.27	0.28
S3934_7976	A	G	0.26	0.17	-0.58	0.98	1.00	0.72
S3955_6073	A	G	0.07	0.08	0.04	0.18	0.06	0.06
S3959_18125	G	C	0.22	0.25	0.04	0.93	0.88	0.66
S3968_12525	A	G	0.27	0.25	-0.13	0.00	0.25	0.06
S3969_14479	A	G	0.15	0.14	-0.12	0.00	0.02	0.19
S3978_2991	A	G	0.41	0.46	0.04	0.93	0.46	0.53
S398_40593	T	C	0.10	0.10	-0.02	0.95	0.96	1.00
S3986_419	A	T	0.10	0.11	0.02	0.02	0.08	0.09
S3986_449	T	G	0.09	0.11	0.03	0.00	0.02	0.09
S3991_13145	A	C	0.07	0.09	0.12	0.05	0.13	0.06
S4_125707	G	C	0.07	0.17	0.48	0.95	0.04	0.13
S4_125710	A	T	0.06	0.09	0.21	0.00	0.06	0.03
S401_20838	A	G	0.07	0.12	0.36	0.73	0.00	0.00
S4015_16341	T	G	0.16	0.14	-0.18	1.00	0.87	1.00
S4015_6167	G	C	0.18	0.16	-0.15	0.05	0.02	0.19
S4022_163	A	G	0.40	0.44	0.03	0.89	0.73	0.72
S4032_16373	T	C	0.95	0.53	-0.92	0.50	0.50	0.50
S4061_11610	T	C	0.09	0.11	0.12	0.00	0.10	0.03
S407_61933	T	C	0.15	0.16	-0.02	0.02	0.23	0.19
S4077_13484	A	G	0.08	0.11	0.16	0.64	0.00	0.00
S409_27689	A	G	0.26	0.25	-0.08	0.09	0.13	0.16
S4094_11122	A	G	0.19	0.20	-0.03	0.00	0.15	0.19
S41_33538	A	G	0.25	0.30	0.09	0.00	0.13	0.38
S41_33552	T	C	0.09	0.11	0.01	0.00	0.13	0.00
S41_7278	A	G	0.08	0.09	0.01	0.34	0.00	0.00
S410_60368	T	C	0.03	0.09	0.52	0.95	0.02	0.00
S4112_8692	T	C	0.18	0.22	0.12	0.00	0.06	0.22
S4113_14868	T	C	0.43	0.44	-0.02	0.25	0.35	0.31

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S4129_13988	A	G	0.18	0.18	-0.04	0.02	0.17	0.13
S4132_6168	T	C	0.20	0.19	-0.10	0.02	0.21	0.06
S4132_6187	G	C	0.32	0.21	-0.51	0.93	0.98	0.69
S414_28193	T	C	0.07	0.10	0.32	0.48	1.00	1.00
S414_28199	T	C	0.07	0.08	0.05	0.30	0.00	0.00
S4154_3610	T	C	0.11	0.10	-0.08	0.00	0.02	0.06
S4154_3647	A	G	0.42	0.43	-0.03	0.00	0.69	0.50
S4156_13035	A	G	0.27	0.28	-0.04	0.68	0.81	0.78
S417_23757	A	C	0.46	0.31	-0.42	0.75	0.88	0.63
S417_23803	T	C	0.17	0.16	-0.06	0.02	0.12	0.06
S417_30492	T	C	0.09	0.11	0.12	0.50	0.00	0.03
S4183_17997	A	G	0.19	0.22	0.04	0.00	0.06	0.22
S4186_11024	T	C	0.34	0.29	-0.22	0.77	0.88	0.63
S4186_11035	A	C	0.27	0.21	-0.23	0.93	0.90	0.84
S4186_11043	A	C	0.32	0.26	-0.23	0.80	0.83	0.91
S4188_3560	T	C	0.14	0.17	0.08	0.48	0.06	0.06
S4188_938	A	T	0.17	0.18	-0.01	0.98	0.86	0.94
S419_32315	T	C	0.13	0.13	-0.03	0.00	0.12	0.19
S419_32351	T	C	0.09	0.10	0.01	0.00	0.13	0.00
S419_58324	A	G	0.10	0.12	0.03	0.00	0.08	0.03
S419_58333	T	C	0.07	0.08	0.06	0.18	0.00	0.00
S420_43007	T	C	0.11	0.10	-0.10	0.93	0.90	0.97
S420_43011	A	T	0.79	0.50	-0.67	0.30	0.44	0.38
S420_43015	A	G	0.19	0.17	-0.13	0.10	0.13	0.06
S420_43019	A	G	0.11	0.11	-0.08	0.02	0.04	0.16
S420_43052	A	T	0.87	0.50	-0.81	0.82	0.50	0.50
S420_43056	A	T	0.08	0.08	-0.08	0.89	0.90	0.97
S420_43060	A	G	1.00	0.53	-0.99	0.50	0.50	0.50
S420_43061	A	T	0.48	0.37	-0.33	0.20	0.23	0.22
S420_43062	T	C	0.12	0.11	-0.10	0.02	0.00	0.16
S421_13033	G	C	0.17	0.16	-0.12	0.95	0.85	0.94
S4218_7783	A	G	0.31	0.21	-0.52	0.93	1.00	0.69
S4218_9181	A	T	0.11	0.11	-0.04	0.02	0.06	0.03
S4218_9227	A	C	0.26	0.34	0.16	0.23	0.77	0.78
S4231_6063	A	C	0.99	0.53	-0.99	0.50	0.50	0.50
S4238_9728	A	G	0.04	0.13	0.55	1.00	0.00	0.10
S4260_8800	T	G	0.16	0.20	0.11	0.93	0.87	0.88
S4260_8838	T	C	0.09	0.11	0.09	0.00	0.04	0.03
S4269_11903	G	C	0.33	0.22	-0.60	0.95	1.00	0.60
S4269_12638	T	C	0.17	0.16	-0.12	0.02	0.08	0.03
S4281_1267	T	C	0.06	0.08	0.22	0.73	1.00	1.00
S4308_1512	T	G	0.14	0.15	0.03	0.55	0.04	0.06
S4311_14441	A	C	0.10	0.10	-0.03	0.39	0.00	0.00
S4311_2938	A	G	0.16	0.13	-0.21	0.98	1.00	0.84
S4329_7892	T	C	0.14	0.14	-0.04	0.07	0.06	0.09
S4329_7895	A	G	0.15	0.17	0.03	0.02	0.10	0.19
S4329_7898	G	C	0.21	0.21	-0.08	0.95	0.87	0.81
S433_30916	T	G	0.85	0.49	-0.79	0.59	0.62	0.53
S433_30919	A	C	0.17	0.13	-0.31	0.05	0.00	0.19
S4333_11982	A	T	0.18	0.20	0.03	0.90	0.94	0.84
S4342_17411	A	T	0.08	0.08	-0.04	0.23	0.00	0.06
S4346_4436	A	G	0.11	0.12	0.01	0.11	0.08	0.00
S4346_4446	A	T	0.07	0.08	0.06	0.77	1.00	1.00
S4346_4469	A	T	0.28	0.29	-0.02	0.84	0.83	0.84
S4358_16181	A	G	0.08	0.11	0.16	0.48	0.02	0.03
S437_12758	T	C	0.26	0.32	0.11	0.11	0.25	0.28
S437_12764	T	C	0.06	0.14	0.47	1.00	0.71	1.00
S4383_11071	T	C	0.45	0.45	-0.06	0.07	0.44	0.41
S439_63934	A	T	0.22	0.22	-0.06	0.98	0.88	0.78
S4407_15208	T	G	0.04	0.10	0.40	0.00	0.94	0.97
S442_61980	T	C	0.11	0.13	0.09	1.00	0.96	0.81
S442_61990	A	G	0.16	0.19	0.09	0.05	0.15	0.13
S4423_2020	A	G	0.14	0.14	-0.06	0.64	1.00	0.97
S4423_2064	A	C	0.15	0.16	0.00	0.05	0.10	0.09
S4424_1865	A	G	0.35	0.41	0.09	0.07	0.44	0.34
S4424_1874	T	C	0.12	0.15	0.08	1.00	0.88	0.91
S4424_1892	T	C	0.12	0.14	0.08	0.27	0.06	0.03
S4436_8629	A	G	0.09	0.11	0.04	0.02	0.10	0.09
S4441_1068	A	T	0.15	0.17	0.04	0.02	0.06	0.13

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S4447_17194	G	C	0.15	0.19	0.10	0.64	0.04	0.03
S4454_11543	G	C	0.13	0.15	0.04	0.02	0.04	0.09
S4454_11558	T	G	0.23	0.17	-0.33	0.80	0.65	0.97
S4454_11564	A	G	0.09	0.10	-0.03	0.02	0.04	0.13
S4454_11565	T	G	0.34	0.27	-0.27	0.98	0.81	0.75
S4480_15771	A	G	0.04	0.10	0.40	0.02	1.00	1.00
S4490_10819	A	G	0.17	0.21	0.09	0.07	0.14	0.13
S45_1566	T	C	0.11	0.11	-0.06	0.05	0.12	0.13
S45_1593	A	G	0.16	0.17	-0.01	0.00	0.15	0.09
S45_1599	A	G	0.11	0.11	-0.09	0.00	0.08	0.09
S45_1666	A	G	0.44	0.35	-0.28	0.36	0.27	0.13
S450_9873	T	C	0.20	0.19	-0.12	0.02	0.23	0.06
S4504_16968	A	T	0.15	0.16	0.00	1.00	0.96	0.84
S4506_8720	A	G	0.39	0.46	0.10	0.89	0.58	0.72
S451_29683	T	C	0.08	0.13	0.17	0.86	0.02	0.06
S4512_13020	T	C	0.22	0.28	0.17	0.00	0.77	0.84
S452_14656	A	G	0.48	0.49	-0.04	0.30	0.63	0.37
S455_42126	A	C	0.22	0.16	-0.37	1.00	1.00	0.78
S455_42173	T	C	0.16	0.19	0.04	0.00	0.13	0.16
S4557_11793	A	G	0.06	0.07	0.14	0.32	0.02	0.00
S4576_16665	A	G	0.14	0.17	0.08	0.30	0.02	0.16
S4576_16728	A	G	0.10	0.11	-0.02	0.02	0.04	0.03
S4581_1680	A	G	0.18	0.21	0.06	0.00	0.21	0.03
S46_49059	A	G	0.28	0.28	-0.04	0.00	0.17	0.25
S46_49098	T	C	0.05	0.08	0.30	0.41	0.00	0.00
S46_49113	T	G	0.15	0.15	-0.06	1.00	0.81	0.94
S46_49134	A	T	0.17	0.19	0.02	1.00	0.90	0.91
S460_28007	G	C	0.04	0.10	0.41	0.02	1.00	0.97
S4625_2259	T	C	0.18	0.17	-0.07	0.00	0.12	0.00
S4625_2288	T	C	0.28	0.32	0.05	0.25	0.75	0.78
S4625_3697	A	C	0.19	0.15	-0.21	0.11	0.04	0.22
S4642_10843	T	G	0.09	0.10	-0.01	0.05	0.04	0.07
S4645_14119	G	C	0.14	0.17	0.11	0.75	0.08	0.00
S4645_14169	A	C	0.21	0.22	-0.01	0.91	0.90	0.94
S4659_13059	G	C	0.09	0.12	0.13	0.75	0.00	0.03
S4659_2100	T	C	0.50	0.32	-0.45	0.77	0.94	0.56
S466_22364	T	C	0.07	0.08	0.14	0.32	0.00	0.00
S4684_11376	T	C	0.07	0.07	-0.05	0.25	0.00	0.00
S47_41655	A	C	0.12	0.12	-0.02	0.00	0.12	0.09
S47_92636	T	C	0.09	0.10	0.01	1.00	0.87	0.97
S471_52925	T	G	0.11	0.11	-0.07	0.02	0.10	0.13
S472_62655	A	G	0.06	0.11	0.27	0.91	0.00	0.00
S472_62676	T	C	0.11	0.12	0.04	0.00	0.04	0.06
S4732_13647	A	G	0.27	0.27	-0.03	0.00	0.15	0.13
S4733_11124	T	C	0.17	0.19	0.02	0.86	0.94	0.88
S4733_11160	T	C	0.42	0.47	0.07	0.55	0.46	0.38
S474_29940	T	C	0.30	0.35	0.09	0.00	0.25	0.34
S4742_1143	T	C	0.06	0.07	0.06	0.36	0.00	0.00
S4745_15871	T	C	0.15	0.16	0.04	0.05	0.19	0.16
S4745_15882	A	C	0.16	0.16	-0.07	0.00	0.13	0.09
S4748_9816	A	G	0.13	0.15	0.04	1.00	0.90	0.88
S4762_15967	A	G	0.08	0.10	0.19	0.48	0.00	0.00
S4762_15980	T	G	0.09	0.11	0.11	0.00	0.04	0.13
S477_30357	G	G	0.09	0.11	0.04	1.00	0.04	1.00
S477_57003	T	G	0.11	0.11	-0.01	0.00	0.02	0.13
S478_25056	T	G	0.14	0.13	-0.11	1.00	0.85	0.88
S478_25061	A	G	0.15	0.09	-0.47	0.50	1.00	1.00
S479_51377	A	G	0.11	0.13	0.04	0.98	0.88	0.91
S479_51403	T	C	0.14	0.15	0.01	1.00	0.92	0.97
S479_62565	T	C	0.09	0.10	-0.03	0.00	0.00	0.06
S4803_7713	T	C	0.45	0.43	-0.09	0.73	0.63	0.69
S484_60579	T	C	0.14	0.20	0.19	0.00	0.87	0.88
S485_3485	T	G	0.21	0.20	-0.11	0.02	0.10	0.06
S485_3486	T	C	0.30	0.25	-0.21	1.00	0.87	0.88
S4851_1241	A	G	0.13	0.14	0.04	0.00	0.10	0.03
S4851_1256	A	G	0.15	0.17	0.05	0.00	0.13	0.09
S4858_584	T	C	0.16	0.16	-0.09	0.98	0.94	0.88
S4858_623	G	C	0.10	0.10	-0.10	0.02	0.06	0.00
S4858_631	T	C	0.10	0.10	-0.09	0.11	0.02	0.09

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S4864_15381	T	C	0.19	0.26	0.22	0.84	0.81	0.81
S4868_15487	T	C	0.07	0.10	0.16	0.27	0.00	0.00
S4868_15498	T	C	0.13	0.19	0.24	0.91	0.94	0.90
S4873_538	A	G	0.16	0.15	-0.09	0.98	0.85	0.91
S4887_13230	T	C	0.15	0.15	-0.06	1.00	0.88	0.84
S4887_13261	A	C	0.06	0.10	0.26	0.84	0.00	0.00
S4889_9133	G	C	0.16	0.16	-0.06	0.05	0.04	0.09
S49_44932	A	G	0.18	0.17	-0.07	0.02	0.08	0.25
S490_34259	A	T	0.02	0.09	0.53	1.00	0.00	0.00
S4909_8269	T	C	0.12	0.14	0.01	0.00	0.10	0.07
S491_35145	A	G	0.02	0.08	0.53	1.00	0.00	0.00
S492_28692	A	G	0.15	0.19	0.12	0.07	0.13	0.16
S492_28700	T	C	0.06	0.07	0.08	0.23	0.00	0.00
S492_28704	T	C	0.18	0.21	0.07	0.00	0.13	0.06
S4931_9256	A	C	0.05	0.08	0.36	0.29	0.00	0.00
S4931_9273	T	C	0.10	0.11	-0.01	0.00	0.06	0.03
S4938_9736	T	G	0.27	0.22	-0.22	0.77	0.65	0.88
S4939_14030	A	G	0.21	0.20	-0.11	0.00	0.15	0.25
S4949_2248	A	G	0.30	0.32	-0.02	0.11	0.21	0.22
S4949_5799	A	C	0.15	0.12	-0.22	0.95	1.00	0.75
S495_60381	T	C	0.06	0.11	0.28	1.00	0.02	0.03
S4950_13753	T	C	0.14	0.16	0.01	0.43	0.94	0.97
S4961_671	A	G	0.19	0.15	-0.22	0.18	0.27	0.03
S4962_14935	A	G	0.03	0.10	0.47	0.93	0.00	0.00
S4972_13163	A	G	0.07	0.10	0.23	0.95	0.90	0.97
S4972_13217	A	G	0.09	0.10	0.05	0.93	0.98	0.94
S4972_13233	T	G	0.08	0.10	0.03	0.05	0.08	0.03
S499_10637	G	C	0.07	0.08	0.06	0.75	1.00	1.00
S499_10662	G	C	0.10	0.13	0.08	0.41	1.00	1.00
S4990_13903	T	C	0.15	0.16	-0.05	0.89	0.96	0.97
S4990_3623	T	C	0.14	0.17	0.06	1.00	0.88	0.78
S4992_5064	A	G	0.39	0.42	0.02	0.02	0.54	0.47
S4994_4615	A	G	0.39	0.45	0.08	0.66	0.71	0.69
S5_202601	T	C	0.14	0.15	0.02	0.02	0.13	0.09
S5002_10344	A	G	0.08	0.10	0.11	0.02	0.04	0.03
S5003_5420	A	C	0.16	0.17	-0.05	0.05	0.16	0.19
S5003_5455	A	G	0.11	0.11	-0.07	0.00	0.06	0.03
S501_14607	T	C	0.11	0.11	-0.04	0.00	0.06	0.09
S5016_9963	T	C	0.36	0.40	0.04	0.02	0.37	0.41
S502_41655	A	G	0.14	0.14	-0.07	0.00	0.12	0.07
S5020_4971	T	C	0.55	0.48	-0.23	0.55	0.56	0.63
S5023_4971	A	G	0.13	0.15	0.02	1.00	0.92	0.88
S5041_11517	T	C	0.33	0.38	0.07	0.30	0.75	0.84
S5044_15279	T	C	0.07	0.11	0.19	0.00	0.15	0.07
S5048_88	A	C	0.09	0.11	0.04	0.14	0.06	0.03
S5048_94	T	C	0.11	0.13	0.04	0.57	0.98	0.97
S5048_9524	T	C	0.25	0.22	-0.16	0.09	0.13	0.09
S5048_9525	A	G	0.11	0.10	-0.09	0.07	0.08	0.00
S5048_9528	G	C	0.27	0.23	-0.17	0.95	0.92	0.90
S5048_9533	T	C	0.39	0.31	-0.27	0.86	0.75	0.81
S5048_9534	T	C	0.10	0.10	-0.08	0.05	0.08	0.09
S5048_9569	A	T	0.14	0.13	-0.13	1.00	0.88	0.94
S5048_9571	A	G	0.60	0.41	-0.47	0.07	0.35	0.47
S506_37031	T	C	0.05	0.08	0.16	0.86	1.00	0.97
S506_37071	A	G	0.25	0.26	-0.05	1.00	0.81	0.84
S506_37079	A	G	0.31	0.33	-0.01	0.00	0.38	0.16
S507_54581	A	G	0.06	0.08	0.11	0.29	0.00	0.03
S507_54612	G	C	0.17	0.18	-0.03	0.00	0.10	0.22
S507_54619	A	C	0.10	0.11	0.04	0.02	0.02	0.06
S5082_7150	T	C	0.08	0.13	0.23	0.68	0.00	0.03
S5087_13743	A	G	0.13	0.17	0.09	0.05	0.92	1.00
S510_32002	T	C	0.11	0.12	-0.01	0.86	0.94	0.94
S510_32804	A	T	0.08	0.10	0.14	0.18	0.00	0.00
S5124_840	T	C	0.13	0.14	0.06	0.00	0.12	0.06
S5124_871	T	G	0.18	0.13	-0.40	1.00	1.00	0.78
S514_48213	T	C	0.23	0.23	-0.07	0.00	0.15	0.25
S5145_627	T	C	0.36	0.37	-0.03	1.00	0.56	0.47
S515_51402	T	C	0.17	0.27	0.24	0.07	0.92	0.88
S5169_13231	T	C	0.02	0.08	0.65	0.98	0.00	0.00

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S5175_6450	A	G	0.08	0.12	0.18	0.16	0.96	0.97
S518_32233	A	G	0.12	0.13	0.00	0.02	0.08	0.09
S52_108673	G	C	0.38	0.42	0.04	1.00	0.46	0.59
S5216_1749	T	C	0.13	0.15	0.03	0.24	0.02	0.09
S5234_13885	A	C	0.99	0.53	-0.98	0.55	0.50	0.50
S5234_13895	A	G	0.16	0.15	-0.13	0.20	0.12	0.06
S5234_13897	A	G	0.90	0.52	-0.84	0.41	0.42	0.50
S5234_13912	A	G	0.14	0.13	-0.11	0.00	0.08	0.03
S5234_13937	T	G	0.13	0.12	-0.10	0.00	0.08	0.03
S5237_914	T	C	0.69	0.46	-0.53	0.93	0.42	0.38
S5237_923	T	C	0.18	0.17	-0.14	0.00	0.08	0.13
S5247_13878	T	C	0.27	0.39	0.24	0.43	0.19	0.16
S5247_13892	A	C	0.06	0.10	0.25	0.07	0.00	0.00
S5264_4561	A	G	0.17	0.17	-0.08	0.02	0.08	0.06
S5267_9261	A	T	0.24	0.25	-0.02	0.00	0.19	0.09
S53_114922	T	C	0.28	0.29	-0.03	0.00	0.08	0.22
S5300_14031	A	G	0.46	0.45	-0.07	0.00	0.58	0.50
S532_26696	T	C	0.11	0.11	-0.02	0.57	1.00	1.00
S5320_6144	A	G	0.19	0.22	0.06	0.00	0.13	0.13
S534_50342	T	C	0.29	0.30	-0.01	0.77	0.25	0.13
S535_36264	A	T	0.10	0.11	-0.02	0.00	0.12	0.06
S5354_5176	A	G	0.23	0.22	-0.07	0.00	0.04	0.31
S5355_4212	A	G	0.08	0.11	0.10	0.00	0.04	0.16
S5355_4245	G	C	0.34	0.43	0.13	1.00	0.75	0.50
S537_27533	A	G	0.07	0.08	0.03	0.30	0.02	0.00
S5373_12424	A	G	0.10	0.11	0.01	0.24	0.00	0.06
S538_28310	A	G	0.08	0.08	-0.05	0.10	0.08	0.00
S538_28341	T	C	0.73	0.50	-0.55	0.25	0.42	0.47
S5389_14146	A	G	0.49	0.37	-0.35	0.23	0.27	0.25
S5393_11361	A	G	0.13	0.17	0.11	0.32	0.96	0.91
S54_54726	G	C	0.03	0.10	0.45	0.98	0.00	0.03
S5409_13245	A	G	0.18	0.18	-0.09	0.07	0.13	0.06
S5411_1049	A	T	0.12	0.15	0.14	0.48	1.00	1.00
S5411_1090	A	C	0.12	0.13	-0.01	0.00	0.08	0.09
S5422_11710	T	C	0.25	0.20	-0.19	0.09	0.06	0.31
S5435_5870	T	C	0.15	0.17	0.01	0.02	0.02	0.06
S5435_7371	A	G	0.15	0.12	-0.28	1.00	1.00	0.81
S5435_7373	A	G	0.01	0.08	0.66	1.00	0.00	0.00
S544_42916	T	C	0.11	0.13	0.07	1.00	0.96	0.91
S5460_12848	T	C	0.09	0.09	-0.06	0.70	1.00	0.97
S5460_12860	T	C	0.20	0.23	0.04	0.05	0.19	0.06
S5469_1214	A	C	0.34	0.23	-0.47	0.91	1.00	0.66
S5477_7612	A	G	0.35	0.38	0.03	0.27	0.26	0.13
S5498_8121	T	C	0.46	0.46	-0.05	0.16	0.60	0.38
S55_4738	A	T	0.26	0.29	0.04	0.95	0.79	0.88
S55_4775	T	C	0.09	0.10	-0.03	0.02	0.10	0.09
S55_77951	A	T	0.09	0.09	-0.05	0.11	0.06	0.03
S5517_12115	T	C	0.12	0.09	-0.30	0.36	0.00	0.00
S552_27309	A	G	0.40	0.46	0.07	0.16	0.60	0.50
S552_27317	A	C	0.26	0.28	0.01	0.93	0.86	0.90
S552_27411	T	G	0.50	0.48	-0.09	0.39	0.56	0.50
S5520_12071	G	C	0.35	0.38	-0.01	0.80	0.27	0.09
S5520_12107	T	C	0.35	0.34	-0.10	0.30	0.19	0.44
S554_58465	T	G	0.01	0.08	0.77	1.00	0.00	0.00
S5563_4301	G	C	0.15	0.15	-0.09	0.14	0.08	0.06
S5563_4361	T	C	0.20	0.21	-0.02	1.00	0.90	0.91
S5563_8359	A	G	0.18	0.15	-0.12	0.45	0.02	0.00
S5564_12570	T	C	0.32	0.27	-0.20	0.95	0.79	0.84
S5564_12576	A	T	0.20	0.18	-0.14	0.07	0.12	0.13
S5564_12597	T	C	0.25	0.22	-0.17	0.84	0.92	0.94
S5564_12601	T	C	0.42	0.33	-0.29	0.25	0.27	0.16
S5564_7154	G	C	0.19	0.23	0.10	0.02	0.12	0.23
S5564_7159	A	T	0.11	0.13	0.09	1.00	0.90	0.84
S5564_7180	T	C	0.17	0.22	0.17	0.64	0.85	0.91
S5572_1495	A	T	0.07	0.11	0.17	0.98	0.92	0.94
S5579_2892	T	G	0.46	0.26	-0.75	0.93	0.98	0.53
S56_14787	A	G	0.29	0.23	-0.23	0.48	0.13	0.16
S56_53393	T	T	0.05	0.11	0.48	1.00	0.88	1.00
S5605_7586	A	G	0.31	0.32	-0.02	0.27	0.90	0.78

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S5611_6394	G	C	0.06	0.10	0.22	0.30	0.00	0.00
S5626_8666	A	G	0.02	0.08	0.68	1.00	0.00	0.00
S5626_8687	A	G	0.08	0.10	0.08	0.00	0.08	0.13
S5626_8690	T	C	0.13	0.15	0.04	0.00	0.12	0.09
S5628_8172	A	G	0.10	0.11	-0.02	0.00	0.12	0.13
S5628_8179	T	C	0.06	0.11	0.34	0.70	0.00	0.00
S5631_8341	A	G	0.13	0.13	-0.01	0.00	0.15	0.09
S5636_8068	T	G	0.17	0.19	0.04	0.07	0.08	0.09
S5636_8085	T	C	0.34	0.42	0.17	0.00	0.50	0.47
S5637_8382	A	C	0.27	0.28	-0.03	0.29	0.12	0.22
S5637_8434	A	G	0.32	0.34	0.02	0.82	0.79	0.91
S5658_6609	A	G	0.33	0.35	-0.01	0.25	0.21	0.19
S5702_7119	A	G	0.30	0.34	0.07	0.02	0.23	0.25
S5702_7158	G	C	0.07	0.08	0.07	0.69	1.00	1.00
S5705_9087	A	G	0.30	0.34	0.08	0.16	0.21	0.22
S571_30656	T	C	0.31	0.27	-0.19	0.03	0.19	0.22
S571_30659	T	C	0.58	0.44	-0.36	0.43	0.73	0.72
S571_30681	T	C	0.13	0.12	-0.12	0.00	0.10	0.09
S5716_14147	A	G	0.09	0.10	0.02	0.07	0.04	0.00
S572_36519	T	C	0.13	0.12	-0.10	0.45	0.94	0.97
S572_52678	A	G	0.08	0.10	0.05	0.00	0.02	0.13
S5720_6918	T	G	0.15	0.15	-0.03	0.02	0.12	0.03
S5720_6919	A	G	0.10	0.11	0.01	0.95	0.98	0.97
S573_32733	A	C	0.09	0.10	0.01	1.00	0.92	0.91
S573_32737	A	C	0.08	0.14	0.27	0.00	1.00	0.91
S5734_13737	T	G	0.11	0.12	-0.03	0.00	0.10	0.06
S5735_6678	A	G	0.12	0.12	-0.10	0.00	0.17	0.03
S5735_6691	A	T	0.19	0.19	-0.06	0.98	0.96	0.81
S5745_2701	A	C	0.22	0.18	-0.20	0.11	0.02	0.25
S575_52988	A	G	0.10	0.09	-0.17	0.16	0.00	0.06
S575_53001	G	C	0.28	0.24	-0.20	0.00	0.19	0.17
S5753_11842	A	G	0.19	0.18	-0.09	0.09	0.08	0.13
S5757_1091	A	T	0.08	0.12	0.19	0.91	0.02	0.06
S5766_13848	T	C	0.17	0.20	0.06	0.02	0.08	0.16
S5774_13958	A	G	0.17	0.17	-0.05	0.18	0.08	0.10
S5789_2291	T	C	0.14	0.15	0.04	0.00	0.06	0.13
S5789_2293	A	C	0.11	0.16	0.13	0.07	0.98	0.97
S58_133027	T	C	0.20	0.20	-0.07	0.02	0.12	0.09
S581_5760	A	G	0.07	0.09	0.13	0.39	0.00	0.00
S583_11115	A	G	0.09	0.09	-0.02	0.00	0.21	0.00
S583_14760	A	G	0.07	0.08	0.11	0.61	1.00	1.00
S5843_12815	T	C	0.06	0.13	0.43	0.93	0.04	0.06
S5857_11488	T	C	0.18	0.17	-0.09	0.07	0.13	0.13
S5857_11515	A	G	0.10	0.10	-0.08	0.09	0.02	0.06
S5859_12138	A	G	0.34	0.38	0.06	0.00	0.25	0.28
S5860_12734	A	G	0.10	0.09	-0.08	0.23	0.04	0.00
S5862_9099	A	T	0.13	0.14	0.03	1.00	0.88	0.91
S5873_6469	T	C	0.15	0.15	-0.06	1.00	0.92	0.97
S5879_1569	T	C	0.10	0.17	0.30	0.24	0.98	0.97
S5888_11960	T	G	0.12	0.11	-0.08	0.30	0.00	0.03
S5908_5549	T	C	0.19	0.19	-0.04	0.02	0.13	0.09
S5921_2856	T	C	0.12	0.13	0.02	0.55	0.00	0.03
S594_27735	T	C	0.20	0.21	-0.02	0.00	0.15	0.13
S597_13132	A	C	0.04	0.09	0.37	1.00	0.00	0.00
S5973_6260	T	C	0.15	0.15	-0.02	0.02	0.13	0.00
S598_1591	A	G	0.11	0.11	-0.03	0.02	0.08	0.03
S6_161640	A	C	0.07	0.08	0.06	0.32	0.00	0.00
S6_221826	T	C	0.09	0.10	-0.03	0.03	0.06	0.06
S6_221942	T	C	0.12	0.10	-0.24	1.00	1.00	0.97
S6039_1790	T	C	0.16	0.17	0.02	0.00	0.08	0.03
S6039_1821	T	G	0.10	0.10	-0.02	0.93	0.90	0.91
S6039_6257	A	G	0.16	0.18	0.04	0.05	0.13	0.19
S6069_6777	T	C	0.11	0.14	0.13	0.89	0.98	0.97
S6069_6779	A	G	0.15	0.21	0.18	0.00	0.06	0.31
S6069_6781	A	T	0.20	0.29	0.22	0.82	0.73	0.84
S6069_6803	G	C	0.15	0.20	0.17	0.09	0.12	0.09
S6092_12229	A	C	0.08	0.13	0.18	0.91	0.04	0.00
S6092_12242	A	C	0.12	0.11	-0.08	0.00	0.06	0.19
S6139_4643	A	T	0.32	0.37	0.06	0.93	0.48	0.72

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S616_755	A	T	0.40	0.34	-0.22	1.00	0.79	0.79
S6178_11026	T	C	0.12	0.12	-0.11	1.00	0.98	0.91
S6178_11080	A	G	0.22	0.20	-0.14	0.05	0.10	0.16
S6178_11095	A	T	0.36	0.30	-0.21	0.58	0.08	0.22
S6194_7228	T	C	0.42	0.40	-0.10	0.52	0.40	0.19
S6230_5330	T	G	0.08	0.12	0.17	0.02	0.96	0.97
S6232_1574	A	G	0.20	0.19	-0.10	0.95	0.83	0.88
S6232_1583	A	G	0.22	0.22	-0.05	0.00	0.12	0.13
S6232_806	T	C	0.12	0.11	-0.12	0.02	0.00	0.13
S6236_13203	A	G	1.00	0.53	-0.99	0.48	0.50	0.50
S6236_13220	T	G	0.41	0.25	-0.74	0.14	0.00	0.44
S6245_9267	T	C	0.21	0.22	-0.02	0.11	0.04	0.19
S6245_9281	A	T	0.11	0.11	-0.03	0.77	0.92	0.91
S6245_9291	T	G	0.19	0.21	-0.01	0.14	0.08	0.03
S6247_11354	A	G	0.02	0.08	0.65	0.00	1.00	1.00
S6250_6324	T	C	0.26	0.28	-0.04	0.80	0.15	0.06
S626_3128	T	C	0.23	0.26	0.04	0.11	0.87	0.75
S627_25817	T	C	0.12	0.11	-0.11	0.00	0.12	0.09
S627_25845	A	T	0.78	0.49	-0.66	0.68	0.67	0.63
S627_25851	A	G	0.83	0.47	-0.80	0.95	0.50	0.47
S6281_12040	T	C	0.06	0.09	0.13	0.00	0.17	0.03
S6281_12082	A	T	0.09	0.12	0.09	0.00	0.04	0.09
S6281_12094	T	C	0.07	0.08	0.03	0.30	0.00	0.00
S63_23163	A	G	0.22	0.30	0.18	0.02	0.22	0.31
S63_23166	A	C	0.15	0.19	0.14	0.00	0.06	0.03
S6332_2037	A	G	0.16	0.18	0.06	0.48	0.08	0.00
S6332_2050	A	G	0.24	0.23	-0.08	0.05	0.23	0.22
S6350_118	T	C	0.22	0.27	0.11	0.18	0.25	0.23
S636_35716	T	C	0.15	0.14	-0.09	0.00	0.04	0.16
S6413_1993	T	C	0.13	0.16	0.09	0.50	0.04	0.13
S6413_2022	T	G	0.34	0.24	-0.46	0.64	0.69	1.00
S6416_4691	G	C	0.15	0.15	-0.03	1.00	0.90	0.94
S6422_2622	T	G	0.20	0.30	0.26	0.98	0.79	0.81
S6422_2626	T	C	0.15	0.21	0.15	0.02	0.13	0.25
S643_31329	T	G	0.14	0.17	0.03	1.00	0.90	0.94
S6460_6593	A	G	0.47	0.47	-0.07	0.89	0.54	0.31
S6461_2674	A	G	0.03	0.08	0.50	1.00	0.00	0.00
S6511_10737	A	T	0.08	0.09	-0.01	0.23	0.02	0.00
S6511_10752	A	G	0.48	0.47	-0.09	0.18	0.44	0.38
S6513_85	A	C	0.19	0.19	-0.04	1.00	0.87	0.91
S6534_1041	A	G	0.09	0.11	0.07	0.41	0.00	0.00
S6537_1130	T	C	0.11	0.12	-0.02	0.00	0.06	0.03
S6537_1142	T	C	0.21	0.22	0.02	0.95	0.77	0.78
S6568_8142	A	C	0.04	0.09	0.38	0.93	0.00	0.00
S658_36808	A	T	0.21	0.30	0.19	0.98	0.82	0.72
S6581_6737	A	G	0.11	0.11	-0.04	0.00	0.08	0.00
S6597_11805	T	C	0.10	0.10	-0.03	0.00	0.04	0.03
S6597_11829	T	G	0.32	0.31	-0.09	0.43	0.12	0.16
S6605_11342	T	C	0.19	0.22	0.05	0.05	0.19	0.25
S6637_8592	G	C	0.30	0.33	0.01	1.00	0.75	0.78
S6637_8619	A	G	0.22	0.21	-0.06	0.98	0.87	0.97
S6641_8726	T	C	0.13	0.11	-0.14	0.95	0.84	1.00
S6641_8727	A	G	0.12	0.10	-0.14	0.00	0.06	0.06
S6646_2078	G	C	0.22	0.22	-0.03	0.00	0.13	0.19
S6646_6576	T	C	0.30	0.20	-0.57	1.00	1.00	0.56
S6659_483	A	G	0.23	0.24	-0.03	0.07	0.15	0.19
S6659_514	T	C	0.46	0.46	-0.06	0.84	0.58	0.63
S666_12679	A	G	0.20	0.15	-0.37	0.95	1.00	0.78
S668_48978	G	C	0.71	0.43	-0.63	0.66	0.79	0.53
S6685_9333	A	G	0.11	0.11	-0.04	0.00	0.08	0.09
S6685_9361	T	G	0.23	0.25	0.01	0.05	0.25	0.13
S6691_10801	T	C	0.39	0.43	0.04	0.98	0.40	0.22
S6696_5875	T	C	0.23	0.20	-0.16	1.00	0.81	0.88
S6696_5884	A	G	0.24	0.22	-0.15	0.09	0.10	0.13
S6696_5913	A	G	0.21	0.18	-0.15	0.05	0.15	0.16
S67_97681	T	G	0.05	0.11	0.28	0.93	0.00	0.00
S6708_3679	A	G	0.10	0.10	-0.06	0.05	0.04	0.09
S671_15312	A	G	0.13	0.12	-0.05	0.00	0.00	0.09
S671_15332	A	T	0.18	0.22	0.06	0.00	0.62	0.03

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S673_54420	T	C	0.09	0.10	0.06	0.11	0.10	0.03
S673_54444	T	C	0.39	0.42	0.00	0.20	0.21	0.41
S6768_11894	T	C	0.15	0.18	0.04	0.11	0.96	0.97
S6768_11944	A	G	0.15	0.13	-0.18	0.95	0.77	0.97
S677_46769	A	C	0.18	0.19	0.04	0.00	0.33	0.00
S677_47020	A	G	0.15	0.15	-0.08	1.00	0.94	0.91
S6787_5824	G	C	0.11	0.11	-0.09	0.02	0.12	0.09
S6797_11963	A	C	0.11	0.11	-0.07	0.00	0.04	0.06
S6797_11971	T	G	0.08	0.09	0.05	0.30	0.00	0.00
S6798_6040	G	C	0.13	0.13	-0.05	0.02	0.06	0.13
S6798_6049	T	C	0.31	0.31	-0.03	0.20	0.21	0.06
S6802_11818	T	C	0.11	0.13	0.08	0.43	0.96	0.94
S6802_7696	A	C	0.15	0.14	-0.10	0.07	0.12	0.09
S6807_10344	A	C	0.33	0.22	-0.54	0.93	1.00	0.69
S6808_5963	A	G	0.22	0.28	0.11	0.73	0.75	0.97
S6808_6009	A	C	0.10	0.11	0.00	0.00	0.10	0.03
S682_18710	A	G	0.07	0.08	-0.02	0.20	0.00	0.00
S683_22532	A	C	0.22	0.18	-0.21	0.93	0.98	0.81
S683_53108	A	G	0.39	0.40	-0.04	0.77	0.82	0.67
S6869_11899	G	C	0.31	0.31	-0.07	0.55	0.08	0.09
S6869_11911	T	C	0.18	0.20	-0.01	0.07	0.10	0.19
S688_29408	T	C	0.14	0.13	-0.12	0.00	0.12	0.13
S694_13336	T	C	0.29	0.29	-0.09	0.59	0.10	0.22
S694_46708	T	C	0.18	0.19	-0.02	0.98	0.88	0.78
S694_6613	A	G	0.45	0.47	-0.01	0.75	0.67	0.69
S6951_125	A	G	0.29	0.25	-0.20	0.02	0.23	0.25
S6951_153	T	C	0.27	0.23	-0.18	0.05	0.15	0.22
S6951_175	A	G	0.15	0.14	-0.12	0.00	0.12	0.16
S6956_1680	T	C	0.07	0.10	0.26	0.70	0.00	0.00
S6971_9133	A	G	0.13	0.19	0.18	0.16	0.13	0.10
S6971_9137	T	C	0.10	0.13	0.11	1.00	0.94	0.97
S699_13867	T	G	0.37	0.37	-0.06	0.02	0.38	0.25
S699_16261	T	G	0.72	0.44	-0.64	0.70	0.67	0.53
S699_9151	G	C	0.13	0.13	-0.07	0.00	0.06	0.09
S7_86906	T	G	0.10	0.10	-0.03	0.11	0.04	0.17
S7_86913	A	G	0.14	0.14	-0.04	0.05	0.02	0.09
S7_86924	T	C	0.07	0.09	0.18	0.77	1.00	0.97
S7_86930	G	C	0.12	0.12	-0.06	1.00	0.96	0.88
S70_17362	T	G	0.12	0.12	-0.06	0.00	0.08	0.00
S70_63120	A	T	0.17	0.21	0.07	0.07	0.90	0.84
S7003_11185	A	G	0.12	0.13	-0.06	0.09	0.10	0.06
S701_44445	G	C	0.10	0.10	-0.06	0.00	0.12	0.03
S701_44457	T	G	0.41	0.45	0.06	0.02	0.48	0.53
S7010_11935	G	C	0.18	0.19	-0.04	1.00	0.98	0.66
S7016_6792	A	C	0.21	0.20	-0.07	0.05	0.04	0.09
S7018_11327	A	G	0.15	0.15	-0.08	0.02	0.13	0.09
S7031_2426	A	T	0.11	0.14	0.11	1.00	0.85	0.97
S7059_5835	A	G	0.16	0.16	-0.10	0.05	0.04	0.13
S706_41870	T	C	0.21	0.18	-0.17	0.00	0.25	0.09
S706_41884	T	C	0.99	0.53	-0.99	0.50	0.50	0.50
S706_41893	A	G	0.68	0.43	-0.60	0.00	0.46	0.47
S706_41899	T	G	0.10	0.09	-0.11	0.00	0.14	0.06
S7061_11203	T	C	0.26	0.29	0.08	0.00	0.12	0.31
S707_1627	T	C	0.24	0.20	-0.20	1.00	0.85	0.97
S7076_10807	A	T	0.06	0.07	0.06	0.27	0.00	0.03
S7080_2177	A	G	0.09	0.10	-0.01	0.41	0.04	0.00
S7106_610	A	G	0.21	0.25	0.07	0.09	0.10	0.06
S7114_11605	T	C	0.06	0.07	0.06	0.64	1.00	1.00
S7120_2212	G	C	0.15	0.17	0.08	0.98	0.90	0.88
S7120_2254	T	C	0.12	0.15	0.07	0.11	0.14	0.03
S714_8660	A	G	0.07	0.09	0.13	0.33	0.00	0.00
S7164_8123	A	G	0.40	0.43	0.01	0.32	0.37	0.31
S717_16667	T	G	0.08	0.08	-0.05	0.36	0.00	0.00
S717_16733	T	C	0.17	0.16	-0.08	1.00	0.75	1.00
S7180_9429	A	C	0.35	0.24	-0.47	0.69	0.73	0.97
S7180_9731	T	G	0.15	0.13	-0.14	0.00	0.04	0.19
S7180_9769	A	C	0.35	0.28	-0.26	0.93	0.83	0.78
S7180_9778	A	G	0.70	0.48	-0.52	0.69	0.67	0.56
S7184_11781	A	G	0.10	0.11	-0.02	0.00	0.04	0.06

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S7192_7009	A	G	0.10	0.10	-0.04	0.14	0.06	0.03
S7192_7619	T	C	0.10	0.11	0.01	0.89	0.92	0.91
S7199_5559	A	T	0.08	0.12	0.19	0.70	0.00	0.00
S72_106186	G	C	0.25	0.39	0.33	1.00	0.35	0.38
S7201_7631	T	C	0.12	0.12	-0.07	0.02	0.17	0.06
S721_34823	G	C	0.13	0.15	0.04	1.00	0.98	0.72
S7220_3143	A	C	0.15	0.18	0.04	0.68	0.04	0.09
S7220_3172	T	C	0.09	0.11	0.02	0.00	0.12	0.13
S7224_2726	T	C	0.07	0.09	0.14	0.30	0.00	0.00
S7236_10943	T	C	0.25	0.26	-0.01	0.84	0.88	0.88
S725_23744	A	G	0.11	0.16	0.17	0.07	0.92	0.91
S7250_10102	A	G	0.13	0.13	-0.10	0.00	0.12	0.03
S726_26614	A	T	0.12	0.12	0.00	1.00	0.98	0.91
S726_37547	G	C	0.04	0.09	0.33	0.95	0.00	0.03
S7261_6089	A	G	0.42	0.47	0.05	0.43	0.29	0.44
S7273_9246	T	C	0.13	0.15	0.01	0.43	0.00	0.00
S7273_9264	T	C	0.09	0.09	-0.06	0.14	0.00	0.09
S729_11392	G	C	0.39	0.37	-0.11	0.09	0.27	0.34
S729_11401	T	C	0.12	0.11	-0.11	0.09	0.04	0.09
S73_20435	G	C	0.21	0.15	-0.38	0.02	0.00	0.16
S73_85738	A	C	0.11	0.12	0.00	1.00	0.92	0.90
S730_11048	A	T	0.06	0.12	0.36	0.86	0.00	0.06
S7314_1884	T	C	0.16	0.10	-0.39	0.48	0.00	0.00
S7318_8680	A	G	0.16	0.17	0.01	0.14	0.06	0.06
S732_36925	A	G	0.96	0.53	-0.92	0.55	0.52	0.53
S732_36926	T	C	0.11	0.10	-0.09	0.00	0.02	0.03
S732_36957	G	C	0.18	0.17	-0.14	1.00	0.96	0.91
S732_36964	T	C	0.12	0.11	-0.10	0.00	0.08	0.06
S7326_6572	T	C	0.10	0.10	-0.07	0.91	0.96	0.97
S7360_3421	A	G	0.10	0.13	0.06	1.00	0.88	0.75
S7360_3450	A	G	0.13	0.14	-0.02	0.30	0.04	0.03
S7405_8960	A	G	0.05	0.11	0.46	0.14	1.00	1.00
S742_48603	G	C	0.28	0.31	0.04	0.00	0.25	0.25
S743_12254	T	C	0.07	0.12	0.28	0.48	0.00	0.00
S744_45141	A	C	0.11	0.11	-0.01	1.00	0.88	1.00
S7445_2324	T	C	0.07	0.08	0.11	0.18	0.00	0.03
S745_46029	A	G	0.08	0.10	0.06	0.93	0.96	1.00
S745_46044	A	G	0.20	0.25	0.11	0.25	0.90	0.88
S7470_2639	T	C	0.96	0.53	-0.94	0.43	0.50	0.44
S7470_2675	T	C	0.40	0.32	-0.28	0.86	0.71	0.84
S7476_48	A	G	0.15	0.17	0.03	0.65	0.90	0.94
S7476_74	T	G	0.13	0.11	-0.10	0.98	0.96	0.84
S7476_80	A	C	0.04	0.08	0.37	0.43	0.02	0.03
S748_4521	T	C	0.16	0.15	-0.11	0.00	0.12	0.09
S748_4530	T	G	0.80	0.47	-0.74	0.98	0.52	0.53
S7498_3262	A	T	0.15	0.18	0.14	0.98	0.98	0.97
S75_102464	G	C	0.11	0.11	-0.03	0.70	0.94	0.94
S75_15896	A	G	0.38	0.31	-0.26	0.02	0.15	0.25
S75_15898	T	C	0.30	0.26	-0.21	0.05	0.17	0.22
S75_15900	T	C	0.23	0.20	-0.14	0.89	0.83	0.84
S75_15905	G	C	0.14	0.12	-0.17	0.38	0.06	0.00
S75_15906	A	G	0.12	0.11	-0.09	0.05	0.08	0.00
S75_15911	A	G	0.92	0.52	-0.86	0.48	0.50	0.41
S75_15921	T	C	0.12	0.11	-0.09	0.20	0.06	0.09
S75_15922	A	G	0.14	0.12	-0.12	0.11	0.02	0.09
S75_15927	A	T	0.75	0.49	-0.62	0.68	0.60	0.63
S750_2433	A	T	0.15	0.17	0.01	0.61	0.08	0.03
S750_2447	A	C	0.11	0.11	-0.03	0.00	0.10	0.13
S7519_3269	A	G	0.08	0.11	0.15	0.88	0.98	0.91
S752_33458	T	C	0.12	0.13	-0.02	0.05	0.08	0.03
S7560_2557	A	G	0.21	0.25	0.07	0.11	0.19	0.16
S759_46000	T	C	0.11	0.10	-0.10	0.00	0.06	0.03
S759_46003	A	G	0.90	0.52	-0.83	0.41	0.50	0.50
S7592_1441	A	C	0.11	0.10	-0.10	0.00	0.08	0.00
S7592_8689	A	G	0.14	0.16	0.02	0.00	0.08	0.03
S761_44954	A	G	0.41	0.41	-0.04	0.98	0.25	0.25
S762_24921	T	G	0.13	0.15	0.01	0.00	0.15	0.09
S7637_9797	T	C	0.10	0.10	-0.05	0.00	0.08	0.03
S7652_695	A	C	1.00	0.53	-1.00	0.50	0.50	0.50

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S7680_8688	T	C	0.38	0.46	0.11	0.86	0.50	0.38
S7682_2315	A	G	0.12	0.12	-0.08	0.02	0.12	0.00
S7683_313	G	C	0.14	0.14	-0.01	1.00	0.92	0.88
S77_70610	A	C	0.14	0.15	-0.04	0.00	0.21	0.09
S77_70612	A	T	0.07	0.09	0.19	0.32	0.00	0.00
S770_20659	T	C	0.21	0.25	0.10	0.77	0.94	0.97
S7705_6667	T	G	0.16	0.15	-0.10	0.02	0.06	0.06
S7705_6682	A	G	0.17	0.19	0.02	0.98	0.88	0.90
S772_38165	A	G	0.93	0.52	-0.88	0.39	0.50	0.50
S772_38200	T	C	0.13	0.12	-0.09	0.00	0.08	0.06
S7734_2060	T	C	0.10	0.10	-0.02	0.02	0.08	0.06
S7737_10848	A	T	0.02	0.10	0.76	1.00	0.00	0.00
S775_8441	T	C	0.16	0.17	-0.03	0.00	0.12	0.19
S7760_2100	A	G	0.02	0.08	0.66	1.00	0.00	0.00
S7760_7863	A	G	0.12	0.13	-0.02	0.00	0.08	0.13
S7764_451	A	G	0.36	0.42	0.10	0.43	0.29	0.25
S7768_9879	A	T	0.34	0.38	0.03	0.19	0.33	0.28
S78_13532	T	C	0.11	0.11	-0.07	0.00	0.10	0.16
S78_60244	G	C	0.16	0.12	-0.31	0.00	0.00	0.13
S78_65266	A	G	0.06	0.10	0.27	0.16	1.00	1.00
S7801_5440	A	G	0.26	0.28	0.03	0.45	0.04	0.16
S7805_525	A	T	0.38	0.46	0.11	0.09	0.62	0.56
S7805_572	T	G	0.17	0.17	-0.10	0.21	0.06	0.09
S782_13556	T	C	0.09	0.08	-0.04	0.25	0.00	0.00
S7824_1252	T	C	0.43	0.49	0.08	0.73	0.44	0.41
S7825_2328	A	T	0.05	0.10	0.33	0.93	0.00	0.03
S7838_1569	T	C	0.20	0.22	0.01	0.36	0.08	0.03
S7838_1601	A	G	0.08	0.09	0.04	0.77	1.00	0.91
S786_10596	G	C	0.17	0.19	0.01	1.00	0.83	0.88
S7861_7935	T	C	0.33	0.24	-0.45	0.73	0.67	1.00
S787_43008	A	T	0.23	0.24	-0.01	0.00	0.06	0.31
S7874_5509	T	G	0.27	0.37	0.22	0.16	0.25	0.31
S7900_9018	G	C	0.07	0.10	0.24	0.57	1.00	0.97
S7926_2814	A	G	0.10	0.11	-0.05	0.02	0.10	0.03
S7931_3195	A	G	0.20	0.20	-0.03	0.20	0.08	0.13
S7934_3352	T	C	0.12	0.09	-0.35	0.45	0.00	0.00
S7947_9246	T	C	0.41	0.48	0.08	0.14	0.56	0.41
S7947_9247	T	G	0.10	0.10	-0.04	0.00	0.10	0.03
S7967_10025	T	C	0.08	0.08	-0.04	0.30	0.02	0.00
S7967_10049	A	T	0.10	0.11	-0.05	1.00	0.92	0.94
S8_17990	A	G	0.10	0.11	-0.01	0.00	0.12	0.06
S8007_3593	G	C	0.21	0.30	0.25	1.00	0.73	0.66
S801_17522	A	T	0.04	0.10	0.46	0.95	0.00	0.00
S8035_8221	A	T	0.11	0.10	-0.09	0.89	0.94	0.97
S8035_8231	T	C	0.11	0.10	-0.09	0.05	0.06	0.00
S8035_8236	T	G	0.90	0.52	-0.81	0.43	0.52	0.50
S8035_8244	A	T	0.16	0.15	-0.11	0.02	0.04	0.13
S8063_6887	T	C	0.33	0.35	0.00	0.07	0.27	0.31
S8071_20	A	G	0.12	0.13	0.01	0.05	0.12	0.19
S8071_44	G	C	0.09	0.15	0.20	0.87	0.00	0.09
S8077_3907	T	C	0.04	0.10	0.48	1.00	0.00	0.00
S812_24926	A	G	0.28	0.36	0.18	1.00	0.17	0.38
S8161_5562	A	C	0.15	0.13	-0.13	0.95	0.81	0.97
S8169_366	A	G	0.11	0.12	-0.01	0.05	0.21	0.00
S8178_9518	A	G	0.32	0.34	-0.02	0.66	0.75	0.84
S8179_9759	G	C	0.31	0.36	0.09	0.07	0.19	0.19
S8179_9762	T	C	0.13	0.16	0.11	0.00	0.10	0.09
S8189_5604	G	C	0.08	0.12	0.23	0.84	0.00	0.03
S8189_5605	A	G	0.10	0.10	-0.02	0.00	0.08	0.09
S8193_8770	G	C	0.09	0.14	0.14	0.05	0.98	1.00
S8193_8797	T	C	0.17	0.19	0.02	0.00	0.12	0.16
S8194_7179	A	C	0.39	0.43	0.04	0.00	0.63	0.50
S825_42553	T	C	0.34	0.41	0.13	0.00	0.27	0.56
S8262_3649	G	C	0.27	0.27	-0.07	0.00	0.19	0.25
S8267_7232	T	G	0.07	0.07	-0.09	0.75	1.00	1.00
S8267_7244	A	C	0.23	0.24	-0.02	0.07	0.08	0.19
S8267_7248	G	C	0.24	0.28	0.09	0.98	0.81	0.88
S8267_7256	A	G	0.10	0.12	0.05	0.80	0.96	1.00
S827_26443	A	G	0.13	0.12	-0.07	0.02	0.04	0.03

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S828_40355	A	G	0.01	0.07	0.71	1.00	0.00	0.00
S8281_3824	A	G	0.09	0.10	0.04	0.02	0.10	0.00
S8281_3832	A	G	0.16	0.16	-0.04	0.00	0.12	0.22
S8281_3856	T	G	0.10	0.11	-0.05	1.00	0.96	1.00
S8286_1688	A	G	0.12	0.13	-0.01	0.05	0.04	0.03
S8288_1419	T	C	0.11	0.13	0.06	0.16	0.04	0.00
S8288_3234	A	T	0.09	0.11	0.13	0.38	1.00	1.00
S8310_6444	T	C	0.20	0.19	-0.10	0.11	0.06	0.06
S8310_6457	T	C	0.20	0.21	-0.04	0.09	0.12	0.09
S8315_9237	T	C	0.13	0.13	-0.06	0.07	0.04	0.03
S834_32894	T	G	0.12	0.14	0.02	0.00	0.06	0.13
S834_32908	T	C	0.20	0.19	-0.09	0.00	0.10	0.09
S8365_8581	T	G	0.14	0.15	0.01	0.00	0.12	0.09
S8367_1591	T	C	0.03	0.08	0.44	0.07	1.00	0.97
S84_120532	T	C	0.15	0.22	0.26	0.00	0.12	0.19
S84_120547	G	C	0.07	0.09	0.09	0.30	0.00	0.00
S84_120557	T	C	0.07	0.09	0.09	0.16	0.02	0.00
S841_39725	A	T	0.32	0.42	0.19	1.00	0.62	0.72
S841_39770	A	G	0.09	0.10	0.04	0.00	0.10	0.06
S8418_2682	A	G	0.27	0.28	-0.07	0.07	0.19	0.22
S8418_2695	T	C	0.14	0.13	-0.10	0.98	0.90	0.91
S8420_2094	T	C	0.39	0.39	-0.06	0.02	0.23	0.47
S8437_8370	T	G	0.06	0.09	0.22	0.20	0.00	0.06
S8474_9240	A	T	0.21	0.22	-0.01	0.00	0.10	0.13
S85_54175	A	C	0.06	0.07	0.08	0.66	1.00	1.00
S8503_4519	T	C	0.15	0.16	-0.01	1.00	0.94	0.81
S851_8607	A	T	0.02	0.08	0.68	0.00	1.00	1.00
S851_8609	T	G	0.12	0.15	0.07	0.00	0.04	0.06
S851_8623	G	C	0.09	0.09	0.02	1.00	0.87	0.97
S8511_297	T	C	0.10	0.11	0.01	0.98	0.85	1.00
S8511_315	T	C	0.35	0.38	0.01	0.32	0.15	0.38
S8521_1582	T	C	0.01	0.08	0.71	1.00	0.00	0.00
S8534_7416	T	C	0.09	0.07	-0.22	0.32	0.00	0.00
S8534_7417	A	G	0.60	0.42	-0.47	0.48	0.29	0.31
S8537_2842	A	T	0.02	0.08	0.70	0.00	1.00	1.00
S854_2741	A	T	0.10	0.11	0.01	1.00	0.87	1.00
S854_47470	T	C	0.09	0.09	-0.10	0.95	0.92	0.97
S854_47512	T	G	0.10	0.11	-0.04	0.02	0.02	0.13
S8543_4073	T	G	0.05	0.07	0.23	0.23	0.00	0.00
S8555_8733	T	C	0.28	0.31	0.03	0.14	0.21	0.22
S858_23170	A	C	0.41	0.45	0.04	0.16	0.56	0.34
S859_36774	G	C	0.28	0.20	-0.36	0.05	0.02	0.25
S859_36796	A	G	0.17	0.20	0.04	0.02	0.17	0.06
S86_74822	A	G	0.11	0.12	-0.02	0.00	0.04	0.09
S86_74847	T	C	0.41	0.41	-0.06	0.07	0.69	0.75
S8605_3188	A	G	0.12	0.15	0.14	0.07	0.15	0.03
S8605_3221	A	T	0.09	0.13	0.14	0.98	0.88	0.97
S8605_3295	A	C	0.14	0.14	-0.05	0.55	0.02	0.00
S8605_3298	T	C	0.21	0.20	-0.07	0.00	0.19	0.09
S8605_3301	G	C	0.13	0.11	-0.25	0.93	1.00	0.91
S8610_6866	A	C	0.35	0.39	0.02	1.00	0.37	0.72
S8641_2851	A	G	0.46	0.46	-0.05	0.14	0.40	0.38
S8641_8726	A	G	0.09	0.10	0.02	0.70	1.00	0.97
S8646_810	A	G	0.21	0.14	-0.35	0.52	0.98	0.94
S8646_827	T	C	0.13	0.12	-0.11	0.05	0.08	0.09
S8668_8603	A	G	0.35	0.33	-0.11	0.30	0.19	0.13
S869_10302	G	C	0.41	0.44	0.00	0.73	0.65	0.59
S869_10326	T	C	0.13	0.15	0.06	0.00	0.23	0.03
S869_20779	A	T	0.11	0.10	-0.08	0.00	0.02	0.09
S869_20836	A	T	0.02	0.08	0.54	1.00	0.00	0.00
S87_43675	T	C	0.10	0.12	0.04	0.00	0.02	0.09
S87_43696	T	C	0.17	0.18	-0.02	0.00	0.12	0.09
S8715_8578	A	G	0.31	0.32	-0.02	1.00	0.63	0.78
S873_8406	T	C	0.28	0.38	0.17	0.75	0.77	0.69
S873_8415	T	C	0.16	0.18	0.07	0.02	0.27	0.06
S873_8425	G	C	0.14	0.15	0.02	0.05	0.08	0.03
S8735_5866	G	C	0.38	0.42	0.02	0.61	0.21	0.25
S8742_5463	A	G	0.43	0.44	-0.01	0.02	0.50	0.41
S8754_2584	A	C	0.10	0.11	-0.02	0.02	0.08	0.09

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S8765_174	T	G	0.04	0.09	0.39	0.07	1.00	1.00
S8770_7682	A	G	0.18	0.20	0.05	0.11	0.08	0.19
S879_45537	A	G	0.07	0.08	0.09	0.20	0.02	0.00
S8797_1383	A	G	0.10	0.10	-0.08	0.00	0.15	0.06
S880_44671	G	C	0.12	0.13	0.00	1.00	0.90	0.91
S881_16245	A	G	0.05	0.07	0.12	0.73	1.00	1.00
S8818_4593	A	G	0.69	0.48	-0.50	0.50	0.69	0.56
S8818_4630	T	C	0.31	0.23	-0.30	0.91	0.88	0.63
S8837_646	T	C	0.24	0.21	-0.15	0.95	0.77	0.81
S886_8656	T	G	0.44	0.34	-0.30	0.77	0.81	0.81
S886_8671	A	G	0.12	0.11	-0.11	0.00	0.12	0.16
S886_8678	A	G	0.82	0.48	-0.76	0.11	0.42	0.44
S8909_141	T	C	0.12	0.11	-0.08	0.02	0.04	0.00
S8909_95	T	G	0.20	0.18	-0.14	0.02	0.13	0.16
S8909_96	A	T	0.11	0.10	-0.09	0.89	0.98	0.94
S8911_8211	T	C	0.17	0.19	-0.02	1.00	0.90	0.91
S892_29553	T	C	0.15	0.15	-0.04	0.00	0.08	0.16
S892_30012	T	C	0.07	0.12	0.25	0.98	0.06	0.00
S8920_8659	T	C	0.24	0.26	-0.02	0.07	0.90	0.91
S8937_6434	A	C	0.08	0.08	-0.07	0.93	0.92	1.00
S8937_6474	A	T	0.09	0.11	0.02	0.98	0.96	0.94
S8958_1336	T	G	0.24	0.26	0.01	0.91	0.77	0.72
S8958_1360	A	T	0.11	0.12	0.01	0.70	0.96	0.94
S8970_7211	A	G	0.02	0.07	0.53	0.38	0.00	0.06
S9_78967	A	G	0.07	0.13	0.29	0.00	0.96	0.97
S9_80027	A	T	0.19	0.17	-0.14	1.00	0.75	0.94
S9022_1169	A	G	0.14	0.16	0.02	0.00	0.12	0.09
S9034_2606	A	G	0.15	0.15	-0.06	0.05	0.17	0.00
S9044_7097	A	G	0.18	0.19	-0.03	0.05	0.15	0.13
S9069_6156	G	C	0.24	0.25	-0.01	0.39	0.06	0.19
S908_33619	T	C	0.13	0.14	0.01	0.20	0.04	0.16
S9094_7883	T	C	0.21	0.17	-0.24	0.86	0.71	0.97
S9104_4862	A	G	0.10	0.11	-0.02	0.02	0.02	0.06
S9118_6649	A	G	0.39	0.31	-0.27	0.11	0.13	0.22
S9118_6652	T	C	0.12	0.10	-0.15	0.23	0.02	0.00
S9118_6662	T	C	0.17	0.15	-0.12	1.00	0.87	0.91
S9118_6665	T	C	0.43	0.34	-0.32	0.30	0.22	0.22
S9118_6669	A	G	0.17	0.15	-0.14	1.00	0.94	0.88
S9118_6676	A	T	0.26	0.22	-0.18	0.91	0.85	0.88
S9118_6687	G	C	0.70	0.46	-0.57	0.80	0.60	0.66
S9118_6688	A	T	0.27	0.23	-0.20	0.00	0.08	0.06
S9118_6691	A	G	0.18	0.17	-0.12	0.11	0.10	0.13
S9118_6692	T	C	0.37	0.30	-0.25	0.98	0.77	0.72
S9123_7648	A	C	0.06	0.11	0.37	0.57	0.04	0.03
S9127_911	T	C	0.13	0.12	-0.10	0.00	0.06	0.09
S9190_5907	A	G	0.14	0.15	-0.04	0.02	0.21	0.16
S92_44985	A	C	0.09	0.09	-0.03	0.61	0.00	0.00
S9229_274	A	G	0.15	0.18	0.06	0.57	0.04	0.13
S9229_469	T	C	0.33	0.37	0.02	0.36	0.31	0.19
S9244_884	A	G	0.09	0.14	0.19	0.05	0.06	0.03
S9244_904	T	C	0.16	0.22	0.16	0.09	0.08	0.09
S9245_3717	G	C	0.21	0.22	-0.06	0.14	0.13	0.25
S9245_3741	A	G	0.18	0.21	0.04	0.48	0.90	0.91
S9245_3749	G	C	0.13	0.11	-0.22	0.98	1.00	0.88
S9245_3758	G	C	0.64	0.45	-0.49	0.41	0.35	0.38
S9245_3765	G	C	0.17	0.14	-0.19	0.86	0.98	0.78
S9277_2380	T	C	0.50	0.37	-0.37	0.00	0.31	0.31
S9277_2397	A	G	0.67	0.44	-0.55	0.95	0.60	0.72
S9277_2421	T	C	0.54	0.42	-0.35	0.16	0.33	0.34
S9286_6253	A	C	0.35	0.28	-0.27	0.93	0.71	0.91
S9286_6774	T	C	0.11	0.13	0.08	0.00	0.08	0.06
S93_119037	G	C	0.15	0.21	0.15	0.93	0.21	0.09
S93_41312	A	G	0.12	0.11	-0.10	0.00	0.02	0.22
S931_1800	T	C	0.05	0.12	0.32	0.98	0.04	0.03
S9315_4373	A	C	0.31	0.23	-0.24	0.95	0.96	0.78
S9315_4386	A	C	0.44	0.33	-0.34	0.86	0.63	0.91
S9328_234	A	T	0.09	0.11	0.10	0.27	0.00	0.03
S9330_5448	A	G	0.03	0.10	0.49	0.05	1.00	0.97
S9341_868	A	G	0.11	0.12	-0.01	0.02	0.04	0.03

Appendix 4-3: (continued)

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S9341_898	A	G	0.07	0.10	0.14	0.57	1.00	1.00
S94_11259	T	G	0.77	0.47	-0.69	0.68	0.71	0.50
S94_5915	T	C	0.11	0.10	-0.06	0.07	0.02	0.13
S94_9088	A	G	0.15	0.18	0.07	0.00	0.17	0.13
S94_9118	T	C	0.16	0.17	0.00	0.50	0.02	0.06
S9409_1558	T	C	0.48	0.48	-0.05	0.55	0.44	0.28
S9416_2001	A	G	0.16	0.17	-0.02	0.89	0.87	0.94
S942_9301	A	G	0.05	0.10	0.42	0.00	1.00	0.97
S9447_678	T	C	0.08	0.11	0.20	0.05	0.04	0.09
S945_45109	A	G	0.47	0.45	-0.12	0.18	0.35	0.34
S9453_6751	G	C	0.14	0.16	0.04	0.07	0.12	0.03
S9455_1724	A	G	0.10	0.13	0.13	0.05	0.02	0.09
S9455_1735	A	G	0.08	0.10	0.06	0.50	0.00	0.00
S9455_1754	T	C	0.14	0.20	0.21	0.09	0.12	0.07
S9455_1757	A	G	0.09	0.12	0.09	1.00	0.90	0.94
S9470_7375	T	C	0.14	0.15	0.02	0.02	0.08	0.19
S9470_7380	T	G	0.09	0.11	0.10	0.09	0.06	0.09
S95_87070	A	C	0.18	0.13	-0.40	1.00	1.00	0.88
S95_87113	T	C	0.35	0.37	-0.01	0.48	0.25	0.28
S95_88992	T	C	0.37	0.44	0.12	0.59	0.42	0.16
S95_89034	A	C	0.23	0.23	-0.05	0.25	0.08	0.03
S9515_3796	A	G	0.40	0.43	0.01	0.27	0.21	0.34
S9533_692	A	G	0.08	0.11	0.09	0.25	0.00	0.13
S9533_722	T	C	0.16	0.17	-0.02	0.00	0.02	0.16
S9534_6834	A	G	0.18	0.21	0.05	0.02	0.06	0.09
S9534_6845	T	C	0.10	0.11	0.00	0.82	0.88	0.97
S9541_2498	T	C	0.18	0.21	0.07	1.00	0.85	0.78
S9541_2508	A	G	0.09	0.09	-0.05	0.86	0.94	0.97
S9544_7942	A	G	0.42	0.42	-0.04	0.86	0.40	0.44
S9550_8050	A	T	0.30	0.33	0.01	0.00	0.38	0.34
S961_42526	A	G	0.23	0.24	-0.03	0.05	0.92	0.84
S961_4975	T	G	0.38	0.35	-0.13	0.84	0.63	0.72
S9624_2702	A	G	0.13	0.13	-0.07	0.09	0.12	0.06
S9634_1138	T	C	0.16	0.16	-0.05	0.98	0.94	0.94
S9634_1147	T	G	0.19	0.20	-0.04	0.14	0.10	0.09

Locus	A1	A2	Ho	uHe	F	*fu*	*oc*	*bi*
S9634_1184	A	G	0.15	0.17	0.04	0.02	0.15	0.16
S9646_848	A	C	0.07	0.11	0.22	0.86	0.00	0.00
S965_25727	A	C	0.26	0.22	-0.18	0.14	0.23	0.13
S965_25735	T	C	0.97	0.53	-0.95	0.64	0.50	0.50
S965_25755	A	T	0.52	0.39	-0.38	0.34	0.33	0.28
S965_25757	T	C	0.19	0.17	-0.17	0.00	0.23	0.06
S9657_6079	A	G	0.17	0.17	-0.06	0.70	0.90	0.87
S9672_1872	T	G	0.14	0.11	-0.18	0.86	0.98	0.91
S9672_1889	A	C	0.77	0.49	-0.65	0.61	0.58	0.63
S969_15961	A	G	0.22	0.31	0.17	0.91	0.10	0.13
S969_41148	A	C	0.30	0.21	-0.52	0.05	0.00	0.25
S97_53125	G	C	0.12	0.11	-0.10	0.98	0.98	0.88
S971_13005	G	C	0.10	0.09	-0.10	0.05	0.02	0.03
S971_13057	A	C	0.09	0.10	-0.01	0.00	0.06	0.06
S9713_2545	A	G	0.11	0.12	-0.02	0.77	0.94	0.94
S972_32454	T	C	0.42	0.42	-0.05	0.02	0.44	0.38
S9734_6823	T	C	0.16	0.18	0.02	0.93	0.90	0.91
S9770_5235	A	T	0.34	0.35	-0.04	0.27	0.21	0.19
S9772_985	T	C	0.29	0.29	-0.04	0.07	0.17	0.16
S98_26822	T	G	0.07	0.12	0.20	0.95	0.00	0.06
S980_39578	A	C	0.12	0.10	-0.21	0.98	1.00	0.84
S9805_2133	T	C	0.27	0.29	0.01	0.02	0.13	0.31
S9805_2157	T	G	0.20	0.20	-0.05	0.00	0.17	0.13
S9805_3564	A	G	0.07	0.08	0.10	0.23	0.00	0.00
S9823_668	A	G	0.18	0.21	0.06	0.05	0.12	0.13
S9823_698	T	C	0.24	0.25	-0.01	0.93	0.87	0.81
S9853_4957	A	G	0.13	0.16	0.08	0.39	1.00	0.91
S986_24284	A	T	0.08	0.10	0.18	0.48	0.02	0.03
S986_24287	A	T	0.17	0.20	0.06	0.89	0.88	0.94
S991_33825	T	C	0.11	0.12	0.01	0.02	0.08	0.00
S991_33846	T	C	0.09	0.09	0.00	0.02	0.08	0.03
S998_29523	A	G	0.13	0.11	-0.21	1.00	1.00	0.94
S998_29529	T	C	0.09	0.14	0.18	0.05	0.96	0.94
S9981_7821	A	G	0.09	0.10	0.03	0.00	0.04	0.00
							oc	

Chapter 5

Differential introgression identifies speciation genes at varying levels of phylogenetic divergence within the spruce budworm, *Choristoneura fumiferana*, species complex

Introduction

The classic view that reproductive isolation dictates species boundaries (Mayr 1969) necessarily assumes the treatment of species as cohesive entities. This view, however, is challenged by a growing body of literature suggesting that hybridization is not only extensive in nature, but also a potential source of novel adaptive variation (Rieseberg 1997; Seehausen 2004; Mallet 2005, 2007; Abbott *et al*. 2013). In recent years, a more genic view of speciation has become firmly established and embraced by evolutionary biologists, whereby speciation can occur despite extensive gene flow throughout the genome (Wu 2001; Rieseberg and Burke 2001; Mallet 2001; Noor 2002; Via and West 2008; Via 2012). Here, species boundaries are maintained at particular genetic regions (or "genomic islands" (Turner *et al*. 2005) or "continents" (Michel *et al*. 2010) of speciation) rather than by genome-wide isolation between species (Wu 2001; Via and West 2008; Via 2012; Nosil and Feder 2012). One hypothesis posits that such genomic islands are characterized by a buildup of linkage disequilibrium in genomic regions tightly linked to loci under divergent selection between species through reductions in gene flow and recombination around these loci (Via and West 2008; Via 2012). Thus, while species may maintain their genomic integrity in these genomic regions, much of the genome remains free to move across species boundaries.

Conveniently, this semi-permeability of genomes can be exploited to identify genes associated with species boundaries. Differential patterns of gene flow in admixed populations

result in heterogeneity in estimates of genetic differentiation (*Fst*) between loci that can be detected through genome scans (Lewontin & Krakauer 1973; Nosil *et al.* 2009; Strasburg *et al* 2012). Loci that are significantly differentiated, relative to neutral levels determined from genome-wide estimates, are then identified as potential targets of selection (Beaumont and Nichols 1996; Beaumont and Balding 2004; Excoffier *et al.* 2009; Foll and Gaggiotti 2008). However, Fst outliers are more likely to show significant genetic differentiation as a result of physical linkage with the actual loci under selection rather than being the causative loci themselves (Beaumont and Balding 2004). Alternative methods based on other measures of divergence can serve to compliment genome scans by detecting more contemporary forms of selection (Strasburg *et al.* 2012; Hamilton *et al*. 2012). Geographic (Szymura and Barton 1986; Barton and Gale 1993; Porter *et al.* 1997; Payseur 2010) and genomic cline analyses (Gompert and Buerkle 2009, 2011; Payseur 2010; Fitzpatrick 2013) using loci with known allele frequency differences between parental sources of hybrid populations, allow characterization of locus-specific patterns of introgression along geographic and genomic admixture gradients. Geographic clines model the change in allele frequencies in a series of populations relative to the geographic distance of each population across the hybrid zone. These often form sigmoidal curves as a result of selection against hybrids (Payseur 2010). Thus, genes that maintain species boundaries in the face of gene flow are often characterized by narrow, step-like clines relative to other loci (Larson *et al.* 2013). Likewise, loci subject to neutral introgression will have wider, shallower clines. Genomic clines, on the other hand, model the change in genotype frequencies relative the distribution of hybrid indices across a hybrid zone (Gompert and Buerkle 2009). This makes it possible to determine whether certain genotypes are over or under represented in

different genomic backgrounds, and thereby allow for inferences to be made on the types and direction of selection acting at species boundaries (Gompert and Buerkle 2009).

Despite the potential for these methods to reveal speciation genes, few studies have investigated patterns of differential introgression at varying levels of phylogenetic divergence among closely related species or within species complexes (Nosil and Feder 2012). Given that speciation is likely to enlist multiple processes as lineages diverge through time and space (Abbott *et al.* 2013; Nosil and Feder 2012), the suites of genes involved in these processes are also likely to change under different temporal and spatial contexts (Via 2012; Beysard and Heckel 2014; Nosil and Feder 2012). Many studies have noted marked differences in the introgression patterns of loci in comparisons of replicate clines between distinct lineages within species (Beysard and Heckel 2014) and between species (Szymura and Barton 1991; Vines *et al.* 2003; Teeter *et al.* 2010; Nolte *et al.* 2009). Still, others have not (Buerkle and Rieseberg 2001; Larson *et al*. 2013). Similarly, recent work using genome scans that incorporate genetic linkage information has revealed that islands of divergence can increase in both number and size (Hohenlohe *et al.* 2010; Renaut *et al*. 2012; Nadeau *et al.* 2013; Powell *et al*. 2013; Gagnaire *et al*. 2013; Andrew and Rieseberg 2013) and reside in different genomic regions (Gagnaire *et al.* 2013; Andrew and Rieseberg 2013) as divergence progresses. Thus, an understanding of the genes operating at successive stages along the speciation continuum is of great interest.

The spruce budworm, *Choristoneura fumiferana* (Clemens), species complex may be a good model for investigating differential introgression as it relates to genetic divergence. The complex is composed of from 8 to 15 species or biotypes native to North American coniferous forests, many of which are significant forest pests (Volney and Fleming 2007; Lumley and Sperling 2011). Three of these species, *C. fumiferana, Choristoneura biennis* (Freeman) and

Choristoneura occidentalis (Freeman), have abutting distributions in western Canada along the eastern foothills of the Rocky Mountains in Alberta. Although species level phylogenetic relationships within the complex remain unresolved (Powell and DeBenedictis 1995; Lumley and Sperling 2010, 2011; Bird 2013), mitochondrial sequence divergence (2.7-2.9%) clearly places *C. fumiferana* as the sister taxon to the other species with the time of divergence likely occurring prior to 2.3 million years ago (Sperling and Hickey 1994; Brower 1994). Alternatively, *C. biennis* and *C. occidentalis* are much more closely related (Sperling and Hickey 1994; Sperling and Hickey 1995; Lumley and Sperling 2010, 2011a, Bird 2013, Chapter 4) and are likely to have diverged following the Wisconsinan Glaciation (Volney 1985; Nealis 2005). Despite clear differences in degrees of divergence, all three species will readily hybridize and produce fertile progeny (Harvey 1997; Nealis 2005). However, the frequency of hybridization and extent of gene flow between the species in nature suggests significant differences in genetic architecture of their species boundaries. Whereas hybridization and allele sharing between *C. biennis* and *C. occidentalis* is quite extensive in nature, few recent generation hybrids have been found between either of these species and *C. fumiferana* (Chapter 4). Here, we examine genetic differentiation and differential introgession to identify loci exhibiting non-neutral patterns of divergence along the spruce budworm phylogeny. We use clines between reference populations of *C. fumiferana* and *C. occidentalis,* and between *C. biennis* and *C. occidentalis*, to represent deep and recently diverged species pairs, and compare patterns between these clines to draw conclusions on the types of selection likely acting at species boundaries between these species.

Methods

Sampling

Recent work identified a hybrid zone between the three spruce budworm species *Choristoneura fumiferana* (Clemens), *Choristoneura biennis* Freeman, and *Choristoneura occidentalis* Freeman in Alberta and British Columbia (see Chapter 4, Figure 4-1). The latter species was recently renamed *Choristoneura freemani* (Razowski 2008), but we retain the name *C. occidentalis* to maintain continuity with the literature available on this species. We use 461 samples previously analyzed across this hybrid zone (see Chapter 4, Table 4-1), and use the same reference populations to represent parental populations of each species. Samples collected from Edmonton, AB (N=14) and Brightsands Lake, SK (N=8), are used as reference populations for *C. fumiferana*; while Bull River, BC (N=9) and Porcupine Hills - South, AB (N=9) are used as reference populations for *C. occidentalis*; and those from Morrissey Creek (N=5) and McBride, BC (N=11) are used as reference populations for *C. biennis.* These populations represent areas outside of the historical area of overlap in the ranges of these species (Shepherd *et al*. 1995), are predominated by each species' respective primary host, and for those of the western species have been verified on the basis of life history traits and mitochondrial ancestry (Sperling and Hickey 1994; Chapter 2). Male moths of each species were either collected by hand or by ultraviolet light- or pheromone-based (95:5 E, Z-11-tetradecenal flexlure (Contech, Delta, BC)) trapping methods at each of the 45 locations. All material was frozen at -70°C prior to DNA extraction.

Markers

Earlier work (Chapter 4) described a set of 2277 high quality single nucleotide polymorphisms (SNPs) for use in the hybrid zone between these species. RNA-free DNA was extracted from the thoraces and legs of 461 samples using DNeasy Blood & Tissue DNA purification kits (Qiagen). Genotyping-by-Sequencing (GBS) libraries were prepared and sequenced for each sample, and the resulting sequence data was then mined for SNPs by comparison to a reference genome for *C. fumiferana* (Spruce Budworm Consortium, 19 April 2011) employing the filtering and selection criteria described in Chapter 4. From this dataset we defined two subsets of loci for use in cline analyses, consisting of: i) loci with large allele frequency differences ($\delta = |p_1 - p_2|$, where p_1 and p_2 are the frequency of allele p in each population) between reference populations for *C. fumiferana* and *C. occidentalis*; and ii) loci with large δ between *C. biennis* and *C. occidentalis* (Appendix 5-1). Large allele frequency differences were defined as those above the 95th percentile (inclusive) of all loci (Shriver *et al*. 1997).

Candidate Loci Under Natural Selection

Locus-specific signatures of natural selection were identified using BayeScan v. 2.1 (Foll and Gaggiotti 2008). BayeScan assumes an island model where allele frequencies in populations are correlated via a common ancestral gene pool, and that allele frequencies will diverge with time due to differing effects of genetic drift (Foll and Gaggiotti 2008). Selection, on the other hand, is assumed to affect individual loci and tightly linked adjacent genomic regions. Thus, for each locus BayeScan estimates the posterior probabilities of two alternative models, one that

includes the effect of selection (model parameters α and β) and the other without (only β) where α and β correspond to the locus-specific and population-specific components of a population's F_{st} coefficient, respectively (Foll and Gaggiotti 2008). Loci under selection are identified as those in which the model with α has the larger posterior probability, and the type of selection can be inferred by the sign of α: when $\alpha > 0$ loci are under diversifying selection, and when $\alpha < 0$ loci are under balancing selection (Foll and Gaggiotti 2008).

BayeScan was run for all 2277 loci, regardless of allele differentials, between reference populations at each of the deep and recent levels of divergence. Analysis of *C. fumiferana* and *C. biennis* reference populations were also done for comparison. Analyses were also run for each species alone to elucidate whether species-specific selective processes were contributing to patterns observed between species. Outlier analyses were run using default BayeScan parameters but using a prior odds for the neutral model equivalent to the number of loci used (*i.e.* 2277). Loci with significant deviation from the neutral model were identified when the log posterior odds for α was greater than one (*i.e.* posterior probability > 0.91) and the false discovery rate (FDR) was less than five percent (*i.e.* $q \leq 0.05$). Outlier analyses were replicated three times to ensure consistency in the results.

Admixture Analysis and Genomic Clines

We used *Introgress* v. 1.2.3 (Gompert and Buerkle 2010) to investigate genomic clines between species represented by each reference population. The genomic cline between *C. fumiferana* (Ed and BL reference populations) and *C. occidentalis* (BR and P2 reference populations) included all 461 moths, whereas only individuals with greater than 95% western

ancestry (Chapter 4) (N=365) were retained for genomic clines analysis between *C. biennis* (Mr and Mb reference populations) and *C. occidentalis*. We quantified genomic admixture as a hybrid index summarizing across loci the proportion of each moth's ancestry to *C. occidentalis* to visualize the distribution of ancestry coefficients in both genomic clines analyses. Multinomial regressions were used to estimate the change in genotype frequencies at individual loci as a function of hybrid index along each cline (Gompert and Buerkle 2009). To detect locus-specific selection, likelihood ratio tests were used to compare the likelihood of each of these regression models with those expected under the null model of neutral introgression (Gompert and Buerkle 2009, Payseur 2010). Due to non-uniformity in allele frequency differentials among the loci, the parametric procedure was employed to calculate the expected distribution of genotypes based on 5000 simulated admixed populations. Significant deviation from neutral introgression was determined by departures from 95% confidence intervals of a distribution of expected log-likelihood ratios generated by an additional 1000 parametric simulations for each locus (Gompert and Buerkle 2009). QVALUE *v.*1.34.0 (Dabney and Storey) was used to adjust significance thresholds to a false discovery rate of 0.05 using the method of Benjamini and Hochberg (1995). We then summarized excesses (+) and deficiencies (-) of homozygous and heterozygous genotypes in the regression models as increases or decreases in each genotype's total probability density relative to that of the 1000 neutral simulations (Teeter *et al.* 2010; Nolte *et al.* 2009; Hamilton *et al*. 2012; Larson *et al*. 2013). This was done to ascertain whether loci displayed patterns of introgression characteristic of directional selection (an excess of one or the other homozygous genotype), and/or under- or overdominance (a deficiency or excess of heterozygotes, respectively).

Geographic Clines

To elucidate how allele frequencies change with geographic distance between reference populations as a result of introgression, we used ClineFit v. 0.2 (Porter *et al*. 1997) to estimate four-parameter geographic cline models. These models estimate cline centre (*c*) and width (*w*) parameters, as well as allele frequencies on each side of the cline (*pL* and *pR*) to allow for markers that do not reach fixation for alternate alleles at cline extremities (Porter *et al*. 1997). The cline between *C. fumiferana* and *C. occidentalis* was defined as the great-circle path between BL and Mt. The great-circle path is the shortest spherical distance between two points given the radius of the earth (6.4 million metres). A geographic cline between *C. biennis* and *C. occidentalis* was not investigated since interactions between these species are not clinal with respect to geography but rather resemble a mosaic (Chapter 4). The function *dist2Line* in *geosphere* v. 1.2-28 (Hijmans *et al.* 2012) was used to find the intersection coordinates of the shortest path from every collection locality to the transect line between BL and Mt, and then *distHaversine* was used to measure the distance from Mt to each point of intersection. Cline parameters were then estimated for 120 loci with significant allele frequency differences between *C. fumiferana* and *C. occidentalis* using 200 annealing steps and 2000 replicates sampled for support with 20 replicates between each sample (Porter *et al.* 1997). Significant differences in locus-specific cline centre and width estimates between loci were determined by non-overlapping two-unit support limits, as in Carling and Brumfield (2009). The correlation between cline centre and cline width for each marker was then tested using a Spearman nonparametric rank correlation (ρ) test in *R* v.3.0.1 (R Core Team 2013) to account for non-normally distributed data, as in Teeter *et al*. 2008, 2010).

Results

Distribution of ancestry coefficients reveals contrasting patterns of admixture

The selection of markers with allele differences (δ) greater than or equal to the 95th percentile of all loci resulted in nearly equal numbers of SNPs for each of the *C. fumiferana* and *C. occidentalis* (n=120; δ=0.93), and *C. biennis* and *C. occidentalis* clines (n=117; δ=0.23). However, despite employing such relaxed thresholds for allele frequency differences between the species, not a single locus was shared among these two comparisons. These locus subsets resulted in drastically different patterns of admixture between these species, with the cline in hybrid indices taking on a step-like shape between *C. fumiferana* and *C. occidentalis*, whereas it was linear and gradual between *C. biennis* and *C. occidentalis* (Figure 5-2).

Oultier Analysis

Significant *Fst* outliers were found in all pairwise comparisons between species, but not between reference populations within species. Strong evidence for selection, as dictated by log-posterior odds values greater than one, was observed for 63 loci in comparisons between *C. fumiferana* and *C. biennis*, 66 loci in comparisons between *C. fumiferana* and *C. occidentalis*, and only 2 loci in comparisons between *C. biennis* and *C. occidentalis* (Appendix 5-3). Additionally, all loci found in comparisons between eastern and western budworm species were distinct from the two loci found between the two western budworm species. All loci had positive α values indicating diversifying selection with no evidence for balancing selection.

Genomic Clines

The number of markers exhibiting non-neutral patterns of introgression and the shapes of genomic clines differed between deep and recently diverged species of spruce budworms, though there were overall similarities in the modes of selection (*eg.* directional, underdominance, and overdominance). Genomic clines between *C. fumiferana* and *C. occidentalis* were highly concordant and steep in contrast with those between *C. biennis* and *C. occidentalis*, and suggest a deficit of heterozygotes between species (Figure 5-3). However, directional selection (*eg.* A>a, Table 5-2) of homozygous genotypes was the most frequent mode of selection along both genomic clines. This mode of selection is evident when an increase (+) or decrease (-) in the total probability of a homozygous genotype is observed relative to neutral expectations. A decrease in the total probability indicates positive directional selection on a homozygous genotype in a foreign genomic background, whereas an increase favors selection of the alternative homozygous genotype (Nolte *et al.* 2009; Larson *et al*. 2013). Of all loci showing non-neutral patterns of introgression along both genomic clines, 43 of 114 (38%) and 18 of 48 (38%) had increased probability of homozygous genotypes of *C. occidentalis* alleles along genomic clines with *C. biennis* and *C. fumiferana*, respectively. Alternatively, 33 of out 114 (29%) and 22 out of 48 (46%) had increased probability of homozygous genotypes of *C. biennis* and *C. fumiferana* alleles in *C. occidentalis* genomic backgrounds, respectively. These results suggests converse patterns in introgression between these species pairs in regard to the number of loci exhibiting increased total probability of *C. occidentalis* homozygous genotypes in foreign genomic backgrounds. However, the proportion of loci with overall increases in the total probability of *C. occidentalis* homozygous genotypes compared to neutral introgression in each comparison was not significantly different from 0.5: *C. biennis* vs. *C. occidentalis* proportion=0.57,

X^2=1.0658, *d.f.*=1, *P*=0.3019; *C. fumiferana* vs. *C. occidentalis* proportion=0.45, X^2=0.225, *d.f.*=1, *P*=0.6353).

The second most common mode of selection was for underdominance (*eg.* Aa-, Table 5-2) of heterozygous genotypes. This is indicated by a decrease in the total probability of the heterozygous genotype at a locus relative to neutral expectations. Underdominance was evident for 43 of 114 (38%) loci and 20 of out 48 (42%) of all loci with significant non-neutral patterns of introgression along the *C. biennis*-*C. occidentalis* and *C. fumiferana*-*C. occidentalis* genomic clines, respectively. This is in contrast to the number of loci with an overdominant (*eg.* Aa+, Table 5-2) mode of selection, particularly between *C. fumiferana* and *C. occidentalis*, where an increase in the total probability of the heterozygous genotype is observed relative to neutral expectations. 41 out of 114 (36%) and 13 of 48 (27%) loci had non-neutral patterns of introgression consistent with overdominance and positive selection on heterozygous genotypes along the *C. biennis*-*C. occidentalis* and *C. fumiferana*-*C. occidentalis* genomic clines, respectively. Again the proportion of loci with significant deviations in heterozygous genotypes relative to neutral introgression with regard to decreases in the total probability did not differ from that expected by chance (*i.e.* 0.5) (*C. biennis* vs. *C. occidentalis* proportions=0.51, X^2=0.0119, *d.f.*=1, *P*=0.9131; *C. fumiferana* vs. *C. occidentalis* proportion=0.61, X^2=1.0909, *d.f.*=1, *P*=0.2963).

Geographic Clines

Coincidence in cline centre (*c*) estimates along the *C. fumiferana*-*C. occidentalis* geographic cline was observed across most markers with a mean distance of 466.2 km (range

427.0-505.4 km) east of Mt in the direction of BL (Figures 5-3 and 5-4, Appendix 5-4). This corresponds with an area between Strawberry Ridge and Red Lodge, in west-central Alberta north of 51° of latitude. Only four loci had support limits that did not overlap those of all other loci, of which three had cline centres positioned west of the mean and one had a cline centre positioned east of the mean (Figure 5-3, Appendix 5-4). However, there appears to be some discordance among cline width (*w*) estimates as 15 loci had support limits that did not overlap those of all other loci. Of these, 13 had cline width estimates narrower than the mean (89.6 km, range 23.3-186.2 km) and two had width estimates wider than the mean (Figures 5-3 and 5-4, Appendix 5-4). Estimates for allele frequencies (*pL* and *pR*) were also as expected with the frequency of *C. fumiferana* alleles always greater at the east side of the cline, and two-unit support limits for these parameters never overlapped. A moderate but significant correlation between *c* and *w* estimates was found among the loci (Spearman's $\rho = 0.58$, $P<0.001$) suggesting that cline width estimates increase asymmetrically in an eastward direction (Figure 5-4).

Discussion

Although the semi-permeability of genomes has been extensively demonstrated (Nosil *et al.* 2009; Payseur 2010; Strasburg *et al*. 2012), relatively few studies have examined genomic heterogeneity and how it relates to the processes implicated at various stages of phylogenetic divergence (Hohenlohe *et al.* 2010; Renaut *et al*. 2012; Powell *et al*. 2013; Gagnaire *et al*. 2013; Andrew and Rieseberg 2013; Beysard and Heckel 2014). Here we have investigated differential patterns of gene flow between spruce budworm species representing recent and deep levels of phylogenetic divergence. In doing so, we have identified loci with putative roles in maintaining

species boundaries and adaptive introgression between these species. Interestingly, we find striking contrasts in the number and identity of loci acting as putative targets of selection, as well as similarities in the predominate modes of selection likely to be operating along each cline despite overall differences in the shapes of genomic and geographic clines between these species.

Patterns of introgression within clines

Genome scans are useful for identifying putative targets of selection in highly differentiated regions of the genome (Beaumont and Nichols 1996; Beaumont and Balding 2004; Excoffier *et al.* 2009; Foll and Gaggiotti 2008). Here, we found many *Fst* outliers between reference populations of *C. fumiferana* and each of *C. biennis* and *C. occidentalis,* but only two loci with elevated genetic differentiation relative to neutral levels between these latter two species (Appendix 5-3). This suggests overall lower levels of divergence between the two western spruce budworm species, as would be expected from their recent divergence (Sperling and Hickey 1994, 1995; Lumley and Sperling 2011; Bird *et al*. 2013). It is important to note, however, that various neutral processes can lead to similar patterns of differentiation (Strasburg *et al.* 2012; Le Corre and Kremer 2012; Lotterhos and Whitlock 2014). Failure to account for hierarchical population structure and demographic history, such as population bottlenecks for example, have been shown to impact outlier identification and can lead to overestimation of the number of loci under selection (Foll and Gaggiotti 2008; Excoffier *et al.* 2009). This is because both have the effect of reducing overall heterozygosity and consequently narrowing the null distribution of *Fst* from which outliers are identified (Excoffier *et al*. 2009). Since there is evidence of long-term gene flow between these spruce budworm species (Chapter 4) and, at least

among the two western species, genetic variation is continuously distributed with little population structure, genetic drift is unlikely to have contributed to significant genetic differentiation between these species. In addition, genetic drift would be more likely to result in elevated levels of genetic differentiation across the genome. However, a marked difference in *Fst* of the statistical outliers in each cline compared to all other loci suggests that alternative processes are acting on these two suites of loci (Appendix 5-3). Finally, mutation-order speciation can result from the chance fixation of different alleles in different populations in response to similar selective pressures in the environment and can also render significant genetic divergence between species (Nosil and Flaxman 2011). However, mutation-order speciation has been shown to be prevented at low levels of gene flow and thus would not likely be a significant cause of the divergence observed here (Nosil and Flaxman 2011).

Genomic cline analysis allowed the detection of loci exhibiting significant deviation from a model of neutral introgression. Overall deviation from the neutral model was evident for 48 out of 120 loci with large allele frequency differences between *C. fumiferana* and *C. occidentalis*, and 114 out of 117 loci between *C. biennis* and *C. occidentalis* (Table 5-2). Many of these were also identified as outliers using the genome scan approach (23 loci had non-neutral patterns of introgression and were F_{st} outliers in genome scans between *C. fumiferana* and *C. occidentalis*, and both F_{st} outliers between *C. biennis* and *C. occidentalis* also had non-neutral patterns of introgression). Directional selection was the predominant mode of selection along both clines, and suggests that it plays a much larger role in shaping species boundaries between these three species than selection acting on heterozygotes. This is surprising given the steep concordant clines observed across most loci in the comparison between *C. fumiferana* and *C. occidentalis*, which would suggest a greater role for underdominance (Figure 5-3). Directional selection may

result from differential assortative mating caused by differences in interspecific cross-attraction of male and female spruce budworms (Sanders 1971; Sanders *et al.* 1977). For instance, the attractiveness of female *C. fumiferana* to conspecific males is reduced in the presence of females of *C. occidentalis*, and males of this latter species experience increased cross-attraction to *C. fumiferana* females (Sanders *et al.* 1977). Thus, this would suggest that populations of immigrating *C. occidentalis* are more likely to secure mates in new territories than those of *C. fumiferana*. Interestingly, a greater proportion of loci showed excesses of homozygous genotypes with *C. occidentalis* alleles than *C. fumiferana* alleles (Table 5-2). This is corroborated by prior work which showed increased support for an asymmetric gene flow model between these species in which gene flow from populations of *C. occidentalis* into those of *C. fumiferana* was greater than in the opposing direction (Chapter 4). In addition, a significant correlation in geographic cline centre and width estimates also points to greater introgression moving in an eastward direction (Teeter *et al.* 2008).

Given that most non-neutral loci show evidence for directional selection across both genomic clines, it is interesting to find that the single most common genomic cline between eastern and western species was one in which the total probability of heterozygote genotypes was significantly less than that predicted under neutral introgression (*i.e.* Aa-, Table 5-2). This suggests strong selection against admixed genotypes at these loci between species in both genomic clines. However, this is unlikely to be due to intrinsic Dobzhansky-Muller incompatibilities that result from negative interactions between alleles evolved in isolation, since post-zygotic barriers to reproductive isolation between these species are weak. Though differences in cross-attraction are known (Sanders 1971; Sanders *et al*. 1977), prior studies done to characterize reproductive isolation between these species *ex situ* have consistently observed a

freely interbreeding system capable of producing interfertile hybrid progeny (Campbell 1958, 1967; Sanders 1977; Harvey 1997). In addition, prezygotic barriers are incomplete as each of the three species in question responds to the same primary sex pheromone constituent (Sander 1971; Sanders 1974). Nevertheless, the large proportion of locus-specific genomic clines (Table 5-2, Appendices 5-1 and 5-2) in which underdominance was a likely mode of selection is highly concordant with the steep geographic clines observed across all loci. This might suggest strong exogenous barriers to gene flow between species leading to disruptive selection for alternative ecological niches, such as the differences in forest types (*eg.* boreal versus montane) that each species inhabits. Alternatively, extrinsic selection could be acting on immigrants leading to selection against migrants in new territories (Nosil *et al*. 2005). For instance, Allee effects in mate-finding are believed to be capable of driving localized extinctions of spruce budworm populations at endemic population densities (Regniere *et al*. 2012), such as what may be present at species range limits and in hybrid zones (Hewitt 1988).

Few loci show evidence for hybrid vigor as would be indicated by a greater total probability of heterozygous genotypes than that of neutral expectations (Table 5-2). This was evident in only 13 of 48 loci and 41 out of 114 loci with non-neutral patterns of introgression along *C. fumiferana*-*C. occidentalis* and *C. biennis*-*C. occidentalis* clines, respectively. Although evidence is lacking to suggest that interactions between these species may confer adaptive benefits in admixed populations, transgressive phenotypes among hybrids have been reported for larval developmental traits, such as post-diapause emergence of larvae (Volney *et al*. 1984), as well as in the egg weight of F1 hybrid females (Harvey 1985, 1997). This suggests that hybridization and overdominance as a mode of selection, although infrequent, may be an important source of adaptive introgression between these species.

These results, and in particular those of the genomic clines analyses between *C. biennis* and *C. occidentalis,* however, should be interpreted with caution as selection and gene flow are not the only factors leading to differential introgression among loci (Payseur 2010). Both genetic drift and incomplete lineage sorting can render identical patterns to those of gene flow (Muir and Schlotterer 2005; Lexer *et al*. 2006; Payseur 2010; Hamilton *et al.* 2012). There very well may be a strong signal of incomplete lineage sorting underlying the observed patterns, as the allele frequency differences observed between the two western species were very low. However, by restricting these analyses to just the most significantly diverged loci between these species, the occurrence of shared alleles as a result of ancestral polymorphism should have been reduced (Hamilton *et al*. 2012). Genomic cline analyses are also particularly susceptible to false-positives due to genetic drift when the size of the admixed population is small, contemporary gene flow is low and the number of generations since hybridization is high (Gompert and Buerkle 2009), all of which may be characteristic of the hybrid zone between *C. fumiferana* and *C. occidentalis*.

Contrasting patterns of differential introgression at different levels of phylogenetic divergence

Although major differences were found in the number of outlier loci between species in this hybrid zone, there was overall similarity in predominant forms of selection acting on each cline (Table 5-2). This is somewhat surprising given the vastly different amounts of divergence that has occurred between these species. Whereas divergence between *C. fumiferana* and *C. occidentalis* most likely occurred in allopatry due to isolated refugia during the Wisconinan Glaciation, that between *C. occidentalis* and *C. biennis* is likely to have occurred in sympatry (Volney 1985; Powell and De Benedictis 1995; Nealis 2005). In addition, molecular divergence

estimates using mitochondrial sequence data indicates a minimum of three million years of divergence between *C. fumiferana* from the western lineage, and less than one million years between *C. biennis* and *C. occidentalis* (Sperling and Hickey 1995; Brower 1994). Contrary to our findings, recent work has demonstrated alternative patterns along the speciation continuum in other systems. Beysard and Heckel (2014) found that non-neutral introgression and restricted gene flow was more prominent at higher divergence levels between lineages of the common vole. Genomic regions with elevated divergence are also known to increase in number and size with divergence (Renaut *et al*. 2012; Nadeau 2013; Powell *et al.* 2013; Andrew and Rieseberg 2013; Martin and Feinstein 2014), and the same regions often undergo repeated selection in different lineages (Hohenlohe *et al.* 2010; Renaut *et al.* 2012; Nadeau *et al*. 2013; Powell *et al.* 2013; Andrew and Rieseberg 2013).

Here, despite using 2277 SNPs sampled from across the genome, we found two mutually exclusive sets of loci with significant allele frequency differences between species pairs representing deep and recent divergences across the spruce budworm phylogeny. Although it is tempting to attribute this to differences in the underlying genetic architecture of reproductive barriers between these species, further clarification will require genetic linkage maps to better identify linkage relationships among loci, as well as experimentation to verify a basis for selection at these divergent loci (Michel *et al.* 2010; Nosil and Feder 2012). Fine-scale studies employing dense locus sampling will often identify more genetically differentiated regions as Fst outlier tests are not designed to handle many loci (Turner *et al.* 2005; Lawniczak *et al*. 2010; Bierne *et al*. 2014). This may be attributed to the fact that the detection of outliers is dependent on empirical estimates of the neutral level of genetic differentiation, which itself is estimated from the loci investigated (Nosil *et al.* 2009; Nosil and Feder 2012; Strasburg *et al*. 2012). Since

loci with low levels of divergence may be linked to more strongly differentiated regions in genomic "islands" (Turner *et al*. 2005; Via and West 2008; Via 2012) or "continents" (Michel *et al.* 2010) of divergence, they may not not represent neutrally evolving regions and consequently bias estimates of the neutral Fst distribution (Nosil and Feder 2012). Genome scan approaches may also be susceptible to false-positives when endogenous and exogenous barriers are coupled, such as when tension zones are correlated with environmental boundaries between species (Bierne *et al.* 2014). Thus, Fst outliers may represent intrinsic reproductive barriers between species rather than selection for local adaptation (Bierne *et al.* 2014). Nevertheless, similar differences in the number of outlier loci at different levels of divergence have been reported in other studies (Andrews and Rieseberg 2013; Martin and Feinstein 2014) and suggest that the genetic architecture of species boundaries need not be consistent among closely related species.

Conclusions

We have demonstrated that the extent of genomic heterogeneity within the spruce budworm species complex is greater between taxa with greater divergence. Comparisons between *Choristoneura fumiferana* and *C. occidentalis* yielded a larger number of loci with greater levels of genetic divergence than comparisons between populations of *C. biennis* and *C. occidentalis*. Remarkably, only two *Fst* outliers were identified between these latter species. However, these differences do no translate into overall differences in the primary modes of selection acting at deep and recent divergences along the spruce budworm phylogeny, as directional selection was predominant in both comparisons. Further work will be required to determine whether these similarities reflect a common underlying genetic architecture of species

differences between these species. Nonetheless, geographic and genomic clines between *C. fumiferana* and *C. occidentalis* are highly concordant among loci, forming steep clines compared to genomic clines between the two western species, and are suggestive of strong selection against hybrids by underdominance. Ultimately, these results are consistent with a large body of literature citing a positive correlation between phylogenetic divergence and the strengthening of reproductive barriers to gene flow.

Table 5-1: Collection locations and sample size.

Location		N	Latitude	Longitude	Elev (m)	Km. Mt
Merritt (BC)	Mt	9	N50 6.6	W120 47.1	600	0
Kettle Valley RA (BC)	KV	10	N49 06.8	W118 58.8	610	59.55
Kelowna (BC)	Kw	10	N49 57.6	W119 33.5	693	68.15
Greenwood (BC)	Gw	9	N49 05.4	W118 40.6	750	77.65
Monte Creek (BC)	Mn	5	N50 39.0	W119 57.0	356	81.23
Kalamalka Lake PP (BC)	KL	5	N50 12.4	W119 16.1	547	99.61
Morrisey Creek (BC)[3]	Mr	5	N56 19.9	W124 33.0	1261	147.27
McBride (BC)[3]	Mb	11	N53 18.0	W120 10.0	726	212.97
Casket Lake (AB)	Cs	8	N53 46.9	W119 55.5	1631	253.28
Beaver Dam Rd (AB)	BD	10	N53 56.6	W119 14.6	1466	301.12
Bull River (BC)[2]	BR	15	N49 28.7	W115 27.4	810	305.72
Canal Flats (BC)	CF	10	N50 07.0	W115 46.1	839	316.52
Elko (BC)	Ek	5	N49 17.5	W115 09.2	886	316.55
Honeymoon Lake (AB)	HL	10	N52 34.5	W117 43.4	1294	318.59
Edgewater (BC)	Ew	10	N50 46.0	W116 11.7	891	321.38
Field (BC)	Fd	9	N51 24.3	W116 28.6	1249	335.45
Numa Falls (BC)	NF	11	N51 07.8	W116 07.5	1411	343.7
Saskatchewan River Crossing (AB)	SX	10	N51 58.3	W116 44.0	1457	347.88
Moberly Tower Rd (AB)	My	8	N53 32.1	W118 01.7	1579	350.13
Sparwood (BC)	Sw	9	N49 43.5	W114 51.1	1248	357.02
Hinton (AB)	Ht	16	N53 22.9	W117 41.6	1046	361.87
Smith Lake (AB)	SL	5	N51 15.6	W115 56.0	1441	362.16
Mount Shark (AB)	MS	9	N50 35.2	W115 21.7	2214	365.46
Coleman (AB)	Cm	8	N49 38.6	W114 31.6	1456	374.25
Beaver Mines (AB)	BM	10	N49 25.0	W114 17.4	1444	379.21
Pocaterra RA (AB)	Pc	5	N50 41.3	W115 06.3	1642	386.62
Plateau Mountain (AB)	PM	10	N50 14.5	W114 35.6	1757	398.23
Maycroft (AB)	Mc	11	N49 50.7	W114 17.0	1451	399.64
Hailstone Butte (AB)	HB	13	N50 10.4	W114 27.7	1955	403.47
Pembina Forks (AB)	PF	14	N52 58.0	W116 36.8	1317	404.98
Ram Falls PP (AB)	RF	13	N52 05.2	W115 52.7	1639	405.97
Nordegg (AB)	Nd	9	N52 28.7	W116 06.6	1346	411.07
Sibbald (AB)	Sb	10	N51 03.1	W114 56.5	1554	414.39

[1]Reference populations for *Choristoneura fumiferana.*
[2]Reference populations for *Choristoneura occidentalis.*
[3]Reference populations for *Choristoneura biennis*.

Table 5-1: (continued)

Location		N	Latitude	Longitude	Elev (m)	Km. Mt
Porcupine Hills - North (AB)	P1	17	N49 52.0	W114 00.4	1592	418.65
Porcupine Hills - South (AB)[2]	P2	11	N49 48.5	W113 57.3	1410	419.43
Longview (AB)	Lv	13	N50 28.6	W114 25.4	1351	420.24
Strawberry Ridge (AB)	SR	10	N51 46.5	W115 14.8	1426	429.9
Bragg Creek (AB)	BC	12	N50 56.9	W114 34.3	1306	432.92
Wolf Lake Road (AB)	WL	6	N53 29.4	W116 04.0	962	463.61
Crimson Lake PP (AB)	CL	13	N52 27.1	W115 1.6	971	475.91
Red Lodge PP (AB)	RL	12	N51 56.6	W114 14.2	954	500.72
Eagle Point PP (AB)	EP	16	N53 14.8	W114 52.4	764	523.16
Pembina River PP (AB)	PR	17	N53 36.3	W115 00.2	725	532.46
Edmonton (AB)[1]	Ed	14	N53 32.7	W113 26.2	665	623.62
Brightsands Lake (SK)[1]	BL	8	N53 35.0	W108 54.0	638	902.65

[1]Reference populations for *Choristoneura fumiferana.*
[2]Reference populations for *Choristoneura occidentalis.*
[3]Reference populations for *Choristoneura biennis*.

Table 5-2: Summary of cline analyses (genomic and geographic), and genetic differentiation across all loci showing significant non-neutral patterns of introgression.

Homozygous genomic clines	Heterozygous genomic clines	$N_{Cf\text{-}Co}$	$N_{Cb\text{-}Co}$	c	w	$Fst_{Cf\text{-}Co}$	$Fst_{Cb\text{-}Co}$	Modes of Selection
AA:aa	Aa-	1(6)	2	466.4	90.2	-	-	under
	Aa	3(46)	7(9)	469.1	99.3	0.70	-	neutral
	Aa+	1	0	469.4	88.1	-	-	over
AA:aa-	Aa	2(6)	1	468.6	100.2	0.73	-	A>a
	Aa+	4(7)	7	467.4	98.8	0.72	-	A>a, over
AA-:aa	Aa	0(4)	7	-	-	-	-	a>A
	Aa+	2(5)	10	476.9	104.3	-	-	a>A, over
AA-:aa-	Aa+	2	24	480.1	121.9	-	-	over
AA:aa+	Aa-	14(17)	9	462.5	81.6	0.71	-	a>A, under
	Aa	1(5)	2	466.7	91.2	0.73	-	a>A
AA-:aa+	Aa-	0	5	-	-	-	-	a>A, under
	Aa	1(2)	10	464.1	64.8	-	-	a>A
	Aa+	0		-	-	-	-	a>A, over
AA+:aa	Aa-	4	15	460.1	73.6	0.69	0.23	A>a, under
	Aa	2(3)	2(3)	466.5	91.2	0.72	-	A>a
AA+:aa-	Aa-	0	7	-	-	-	-	A>a, under
	Aa	6(7)	1	460.4	83.5	0.72	-	A>a
	Aa+	4	0	467.9	93.4	0.72	-	A>a, over
AA+:aa+	Aa-	1	5	463.5	91.1	-	0.24	under

Loci grouped according to genomic clines for homozygous and heterozygous genotypes. + and – denote excesses and deficiencies of genotypes relative to neutral levels of introgression; *C. fumiferana* (*Cf*) and *C. biennis* (*Cb*) alleles are denoted as "A" and *C. occidentalis* (*Co*) as "a"; sample sizes for genomic clines in each of the *Cf-Co* and *Cb-Co* comparisons are indicated under $N_{Cf\text{-}Co}$ and $N_{Cb\text{-}Co}$, respectively; parentheses indicate the total number of loci grouped regardless of deviation from neutral introgression; mean geographic cline centre (c) and width (w) (in km) for the *Cf-Co* cline are reported for non-neutral loci and all loci (in parentheses); mean F_{st} of all non-neutral loci that also had significant genetic differentiation in outlier analyses in pairwise comparisons between the three species; modes of selection are neutral introgression, directional selection for (a>A) or against (A<a) *Co* alleles in foreign genomic backgrounds, and under- or over-dominance of heterozygote genotypes.

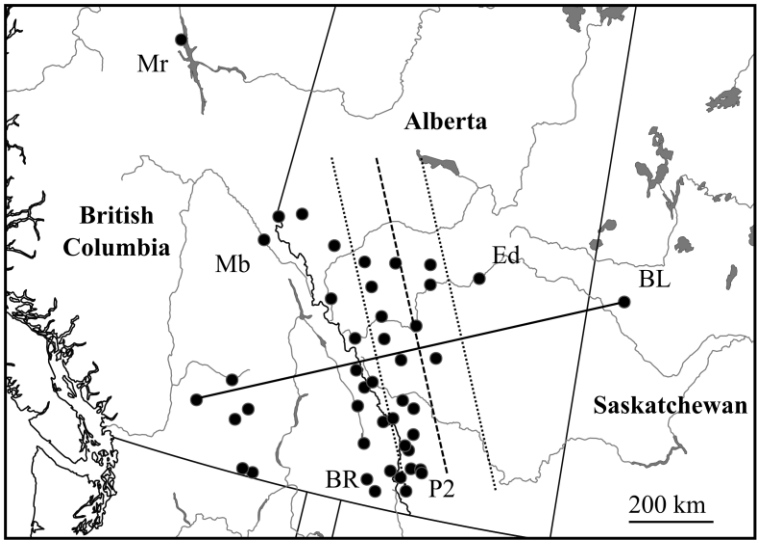


Figure 5-1: Map of the study area showing collection locations. Reference populations are identified as Edmonton (Ed) and Brightsands Lake (BL) for *C. fumiferana*, Bull River (BR) and Porcupine Hills - South (P2) for *C. occidentalis*, and Morrisey Creek (Mr) and McBride (Mb) for *C. biennis.* The geographic cline between *C. fumiferana* and *C. occidentalis* runs from Mt to BL. Boundaries of the area of hybridization between *C. fumiferana* and *C. occidentalis*, as deduced by geographic clines, are indicated by perforated lines, and the geographic cline centre by a dashed line.

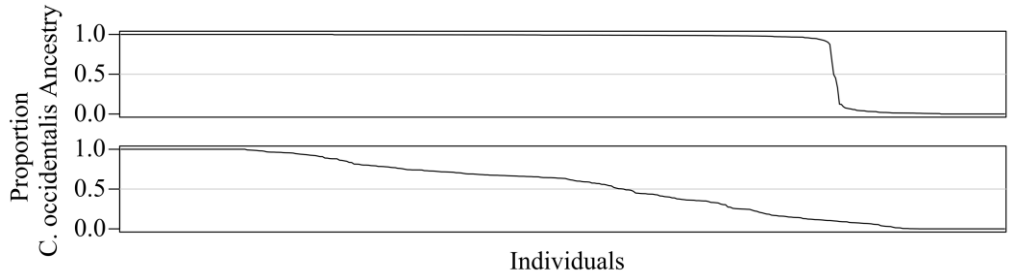


Figure 5-2: Distribution of hybrid indices or ancestry coefficients across genomic clines between *C. fumiferana* and *C. occidentalis* (n=461, top), and *C. biennis* and *C. occidentalis* (n=365, bottom).

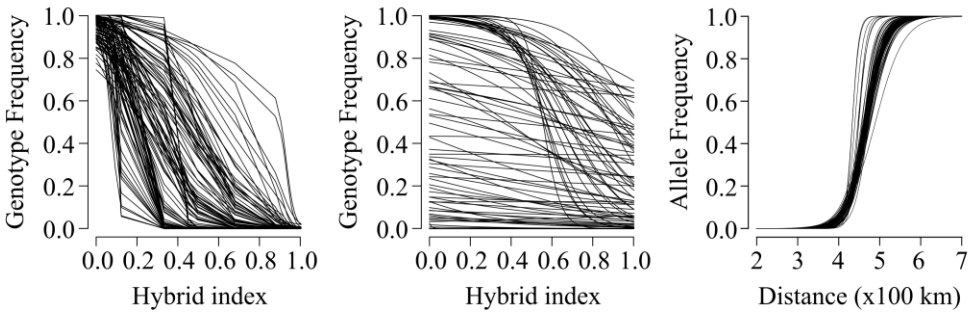


Figure 5-3: Locus-specific genomic and geographic clines. Genomic clines are plotted as the frequency of the *Choristoneura fumiferana* (left) and *C. biennis* (middle) homozygote genotypes against hybrid index (1 = *C. occidentalis*). Geographic clines are plotted as the frequency of *C. fumiferana* alleles against distance from Merritt (BC).

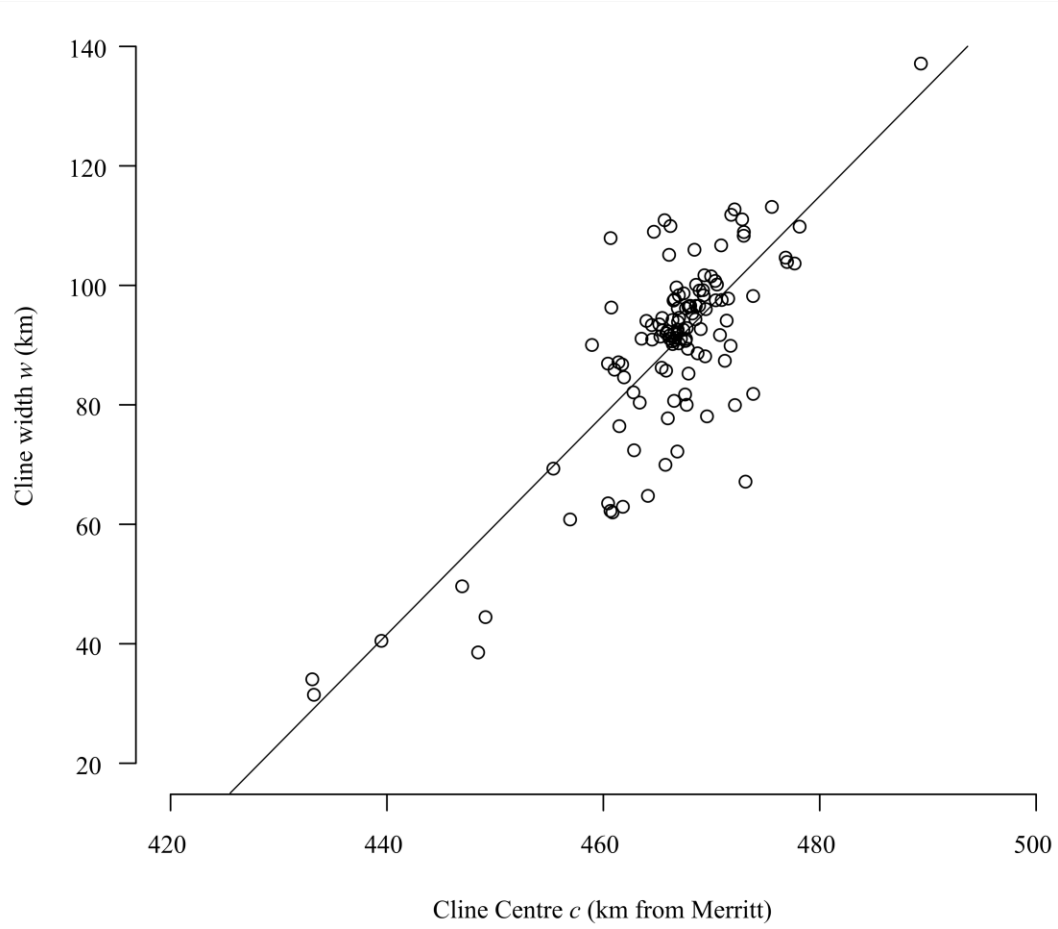


Figure 5-4: Correlation of locus-specific geographic cline width (*w*) and centre (*c*) estimates for 120 loci with significant allele frequency differences between *Choristoneura fumiferana* and *C. occidentalis*.

Literature Cited

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Most, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice., A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., stelkens, R., Szymura, J.M., Vainola, R., Wolf, J.B.W., and D. Zinner. 2012. Hybridization and speciation. Journal of Evolutionary Biology, 26: 229-246.

Andrew, R.L., and L.H. Rieseberg. 2013. Divergence is focused on few genomic regions early in speciation: incipient speciation of sunflower ecotypes. Evolution, 67-9: 2468-2482.

Barton, N., and K. Gale. 1993. Genetic analysis of hybrid zones. In: R.G. Harrison (ed.) *Hybrid zones and the evolutionary process*. Oxford University Press, Oxford. Pp 13-45.

Barton, N.H., and G.M. Hewitt. 1985 Analysis of hybrid zones. Annual Review of Ecology and Systematics, 16: 113-148.

Beaumont, M.A., and D.J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology, 13: 969-980.

Beaumont, M.A., and R.A. Nichols. 1996. A method for quantifying differentiation between populations at multi-allelic loci and its implications for investigating identity and paternity. Genetica, 96: 3-12.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57: 289-300.

Beysard, M. and G. Heckel. 2014. Structure and dynamics of hybrid zones at different stages of speciation in the common vole (*Microtus arvalis*). Molecular Ecology, 23: 673-687.

Bierne, N., Welch, J., Loire, E., Bonhomme, F., and P. David. 2011. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Molecular Ecology, 20: 2044-2072.

Bird, H.M. 2013. Phylogenomics of the *Choristoneura* fumiferana species complex (Lepidopter: Tortricidae). MSc Thesis, University of Alberta, Edmonton, Canada.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences, 91: 6491-6495.

Buerkle, C.A., and L.H. Rieseberg. 2001. Low intraspecific variation for genomic isolation between hybridizing sunflower species. Evolution, 55: 684-691.

Campbell, I.M. 1958. A genetic study of factors determining fecundity in the genus Choristoneura Lederer (Clem.) (Tortricidae). PhD Thesis, University of Toronto, Toronto, Canada.

Campbell, I.M. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist, 99: 482-486.

Carling, M.D., and R.T. Brumfield. 2009. Speciation in passerina buntings: introgression patterns of sex-linked loci identifying a candidate gene region for reproductive isolation. Molecular Ecology, 18: 834-847.

Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., and M. Robles. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21: 3674-3676.

Danecek, P., Auton, A., Abecasis, G., Albers, C., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics, 27: 2156-2158.

Excoffier, L., Hofer, T., and M. Foll. 2009. Detecting loci under selection in a hierarchically structured population. Heredity, 103: 285-298.

Fitzpatrick, B. 2013. Alternative forms for genomic clines. Ecology and Evolution, doi: 10.1002/ece3.609.

Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics, 180: 977-993.

Gagnaire, P.-A., Pavey, S.A., Normandeau, E., and L. Bernatchez. 2013. The genetic architecture of reproductive isolation during speciation-with-gene-flow in lake whitefish species pairs assessed by RAD sequencing. Evolution, 67: 2483-2497.

Gompert, Z., and C.A. Buerkle. 2009. A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. Molecular Ecology, 18: 1207-1224.

Gompert, Z., and C.A. Buerkle. 2010. INTROGRESS: a software package for mapping components of isolation in hybrids. Molecular Ecology Resources, 10: 378-384.

Gompert, Z., and C.A. Buerkle. 2011. Bayesian estimation of genomic clines. Molecular Ecology, 20: 2111-2127.

Harrell, F.E. Jr. with contributions from Charles Dupont and many others. 2013. Hmisc: Harrell Miscellaneous. R package version 3.12-2. http://CRAN.R-project.org/package=Hmisc.

Harvey, G.T. 1985. Egg weight as a factor in the overwintering survival of spruce budworm (Lepidoptera: Tortricidae) larvae. The Canadian Entomologist, 117: 1451-1461.

Harvey, G.T. 1985. The taxonomy of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): A review. Pp. 16-59 in C.J. Sanders, R.W. Stark, E.J. Mullins, And J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferphagous *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519-536.

Hamilton, J.A., Lexer, C., and S.N. Aitken. 2012. Genomic and phenotypic architecture of a spruce hybrid zone (*Picea sitchensis* x *P. glauca*). Molecular Ecology, doi:10.1111/mec.12007.

Hamilton, J.A., Lexer, C., and S.N. Aitken. 2012. Differential introgression reveals candidate genes for selection across a spruce (*Picea sitchensis* x *P. glauca*) hybrid zone. New Phytologist, doi: 10.1111/nph.12055.

Hewitt, G.M. 1988. Hybrid zones - Natural Laboratories for evolutionary studies. Trends in Ecology and Evolution, 3: 158-167.

Hijmans, R.J., Williams, E.D., and C. Vennes. 2012. geosphere: Spherical trigonometry. R package version 1.2-28. http://CRAN.R-project.org/package=geosphere.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A., and W.A. Cresko. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genetics, 6:e1000862. Doi:10.1371/journal.pgen.1000862.

Jin, X., Ho, T.S., and D.P. Smith. 2008. SNMP is a signaling component required for pheromone sensitivity in *Drosophila*. Proceedings of the National Academy of Sciences, 105: 10996-11001.

Larson, E.L., White, T.A., Ross, C.L., and R.G. Harrison. 2013. Gene flow and the maintenance of species boundaries. Molecular Ecology. Doi:10.111/mec.12601

Lawniczak, M.K.N., Emrich, S.J., Holloway, A.K., Regier, A.P., Olson, M., White, B., Redmond, S., Fulton, L., Appelbaum, E., Godfrey, J., Farmer, C., Chinwalla, A., Yang, S.-P., Minx, P., Nelson, J., Kyung, K., Walenz, B.P., Garcia-Hernandez, E., Aguiar, M., Viswanathan, L.D., Rogers, Y.-H., Strausberg, R.L., Saski, C.A., Lawson, D., Colins, F.H., Kafatos, F.C., Christophides, G.K., Clifton, S.W., Kirkness, E.F., and N.J., Besansky. 2010. Widespread divergence between incipient *Anopheles gambiae* species revealed by whole genome sequences. Science, 330: 512-514.

Le Corre, V., and A. Kremer. 2012. The genetic diiferentiation at quantitative trait loci under local adaptation. Molecular Ecology, 21: 1548-1566.

Lexer, C., Kremer, A., and R.J. Petit. 2006. Shared alleles in sympatric oaks: recurrent gene flow is a more parsimonius explanation than ancestral polymorphism. Molecular ecology, 15: 2007-2012.

Lewontin, R.C. and J. Krakauer. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics, 74: 175-195.

Lotterhos, K.E., and M.C. Whitlock. 2014. Evaluation of demographic history and neutral parameterization on the performance of *Fst* outlier tests. Molecular Ecology, doi: 10.1111/mec/12725.

Lumley, L.M., and F.A.H. Sperling. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology, 35: 416-428.

Lumley, L.M., and F.A.H. Sperling. 2011. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232-243.

Mayr, E. 1969. The biological meaning of species. Biological Journal of the Linnean Society, 1:311-320.

Mallet, J. 2001. The speciation revolution. Journal of Evolutionary Biology, 14: 887-888.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution, 20: 229-237.

Mallet, J. 2007. Hybrid speciation. Nature, 446: 279-283.

Martin, C.H., and L.C. Feinstein. 2014. Novel trophic niches drive variable progress towards ecological speciation within an adaptive radiation of pupfishes. Molecular Ecology, 23: 1846-1862.

Michel, A.P., Sim, S., Powell, T.H.Q., Taylor, M.S., Nosil, P., and J.L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences, 107: 9724-9729.

Milne, R.I., and R.J. Abbott. 2008. Reproductive isolation among two interfertile Rhododendron species: low frequency of post-F1 hybrid genotypes in alpine hybrid zones. Molecular Ecology, 17: 1108-1121.

Muir, G., and C. Schlotterer. 2005. Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiationg two hybridizing oaks (*Quercus* spp.). Molecular Ecology, 14: 549-561.

Nadeau, N.J., Martin, S.H., Kozak, K.M., Salazar, C., Dasmahapatra, K.K., Davey, J.W., Baxter, S.W., Blaxter, M.L., Mallet, J., and C.D. Jiggins. 2013. Genome-wide patterns of divergence and gene flow across a butterfly radiation. Molecular Ecology, 22: 814-826.

Nealis, V.G. 2005. Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist 137: 584-597.

Nolte, A.W., Gompert, Z., and C.A. Buerkle. 2009. Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. Molecular Ecology, 18: 2615-2627.

Noor, M.A.F. 2002. Is the biological species concept showing its age. Trends in Ecology and Evolution, 17: 153-154.

Nosil, P. 2012. Ecological Speciation. Oxford University Press, University of Oxford, Oxford, United Kindom. 280pp.

Nosil, P., and J.L. Feder. 2012. Genomic divergence during speciation: causes and consequences. Philosophical Transactions of the Royal Society B, 367: 332-342.

Nosil, P., and S.M. Flaxman. 2011. Conditions for mutation-order speciation. Proceeding of the Royal Society of London B, 278: 399-407.

Nosil, P., Funk, D.J., and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology, 18: 375-402.

Nosil, P., Vines, T.H., and D.J. Funk. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution, 59: 705-719.

Payseur, B.A. 2010. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. Molecular Ecology Resources, 10: 806-820.

Porter, A.H., Wenger, R., Geiger, H., Scholl, A., and A.M. Shapiro. 1997. The *Pontia daplidice-edusa* hybrid zone in Northwestern Italy. Evolution, 51: 1561-1573.

Powell, J.A., and J.A. DeBenedictis. 1995. Evolutionary interpretations, taxonomy, and nomenclature. Pp. 217-275 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Powell, T.H.Q., Hood, G.R., Murphy, M.O., Heilveil, J.S., Berlocher, S.H., Nosil, P., and J.L., Feder. 2013. Genetic divergence along the speciation continuum: the transition from host race to species in *Rhagoletis* (Diptera: Tephritidae). Evolution, 67: 2561-2576.

Razowski, J. 2008. Tortricidae (Lepidoptera) from South Africa. 6: *Choristoneura* Hubner and Procrica Diakonoff. Polish Journal of Entomology, 77: 245-254.

Renaut, S., Maillet, N., Normandeau, E., Sauvage, C., Derome, N., Rogers, S.M., and L. Bernatchez. 2012. Genome-wide patterns of divergence during speciation: the lake whitefish case study. Philosophical transactions of the Royal Society B, 367: 354-363.

Rieseberg, L.H. 1997. Hybrid origins of plant species. Annual review of Ecology, Evolution, and Systematics, 28: 359-89.

Rieseberg, L.H., and J.M. Burke. 2001. A genic view of species integration. Journal of Evolutionary Biology, 14: 883-886.

Rogers, M.E., Krieger, J., and R.G. Vogt. 2001. Antennal SNMPs (Sensory Neuron Membrane Proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. Journal of Neurobiology, 49: 47-61.

Sanders, C.J., Daterman, G.E., and T.J. Ennis. 1977. Sex pheromone responses of *Choristoneura* spp. and their hybrids (Lepidoptera: Tortricidae). The Canadian entomologist, 109: 1203-1220.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution, 19: 198-207.

Shriver, M.D., Smith, M.W., Marcini, A., Akey, J.M., Deka, R., and R.E. Ferrell. 1997. Ethnic-affiliation estimation by use of population-specific DNA markers. American Journal of Human Genetics, 60: 957-964.

Sperling, F.A.H. 1994. Sex-linked genes and species differences in Lepidoptera. The Canadian Entomologist, 126: 807-818.

Sperling, F.A.H., and D.A. Hickey. 1994. Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). Molecular Biology and Evolution, 11: 656-665.

Sperling, F.A.H., and D.A. Hickey. 1995. Amplified mitochondrial DNA as a diagnostic marker for species of conifer-feeding *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 277-288.

Strasburg, J.L., Sherman, N.A., Wright, K.M., Moyle, L.C., Willis, J.H., and L. Rieseberg. 2012. What can patterns of differentiation across plant genomes tell us about adaptation and speciation. Philosophical Transactions of the Royal Society B, 367: 364-373.

Szymura, J.M., and N.H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution, 40: 1141-1159.

Szymura, J.M., and N.H. Barton. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata:* comparisons between transects and between loci. Evolution, 45: 235-261.

Teeter, K.C., Payseur, B.A., Harris, L.W., Bakewell, M.A. Thibodeau, L.M., O'Brien, J.E., Krenz, J.G., Sans-Fuentes, M.A., Nachman, M.W., and P.K. Tucker. 2008. Genome-wide patterns of gene flow across a house mouse hybrid zone. Genome Research, 18: 67-76.

Teeter, K.C., Thibodeau, L.M., Gompert, Z., Buerkle, C.A., Nachman, M.W., and P.K. Tucker. 2010. The variable genomic architecture of isolation between hybridizing species of house mice. Evolution, 64: 472-485.

Turner, T.L., Hahn, M.W., and S.V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae*. PLoS Biology, 3:e285.

Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B, 367: 451-460.

Via, S., and J. West. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Molecular Ecology, 17: 4334-4345.

Vines, T.H., Köhler, S.C., Thiel, M., Ghira, I., Sands, T.R., MacCallum, C.J., Barton, N.H., and B. Nürnberger. 2003. The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied taods *Bombina bombina* and *B. variegate*. Evolution, 57: 1876-1888.

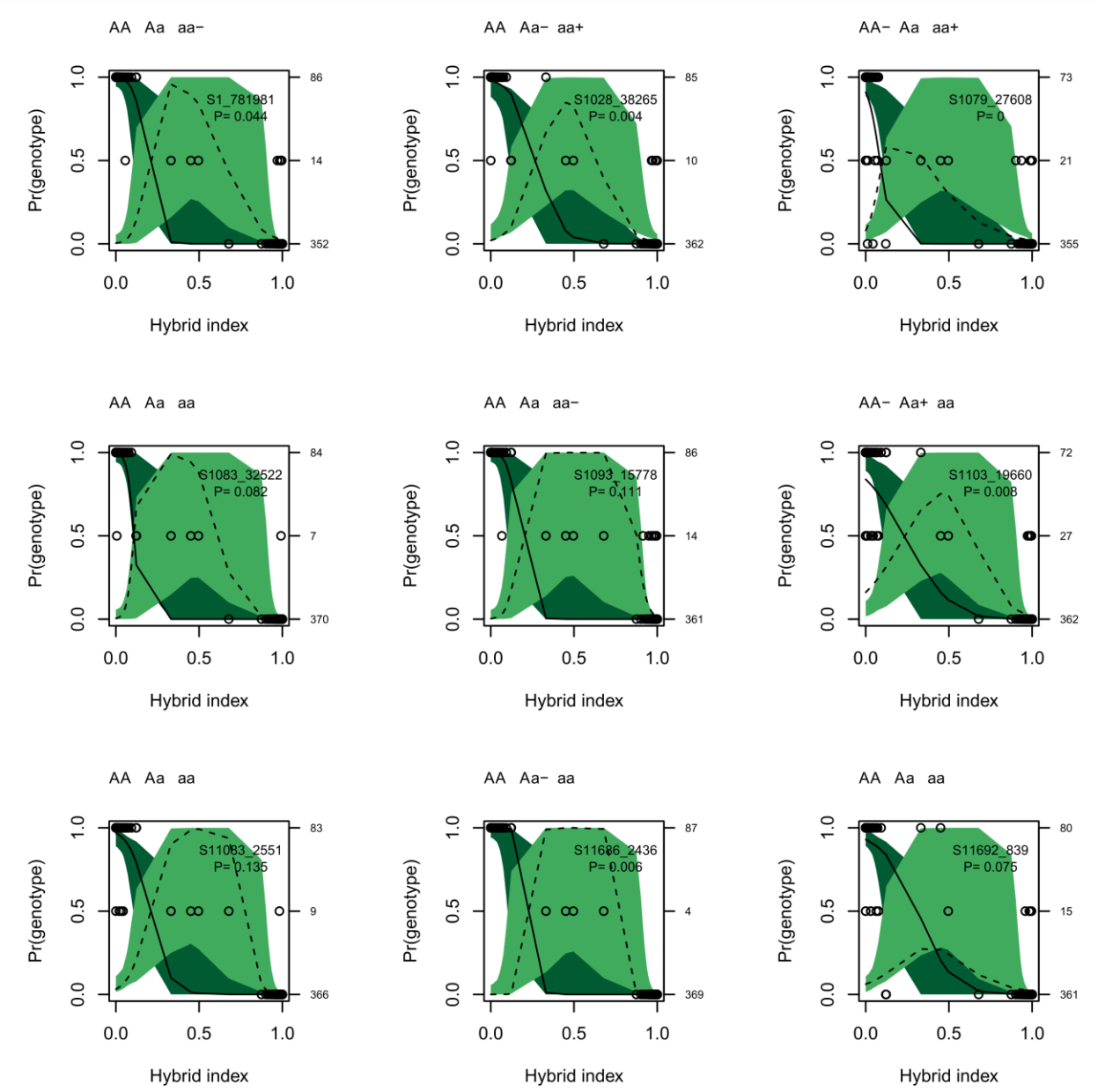
Volney, W.J.A. 1985. Comparative population biologies of North American spruce budworms. Pp. 71-84 in C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characterisitics. Global Change Biology 13:1630-1643.

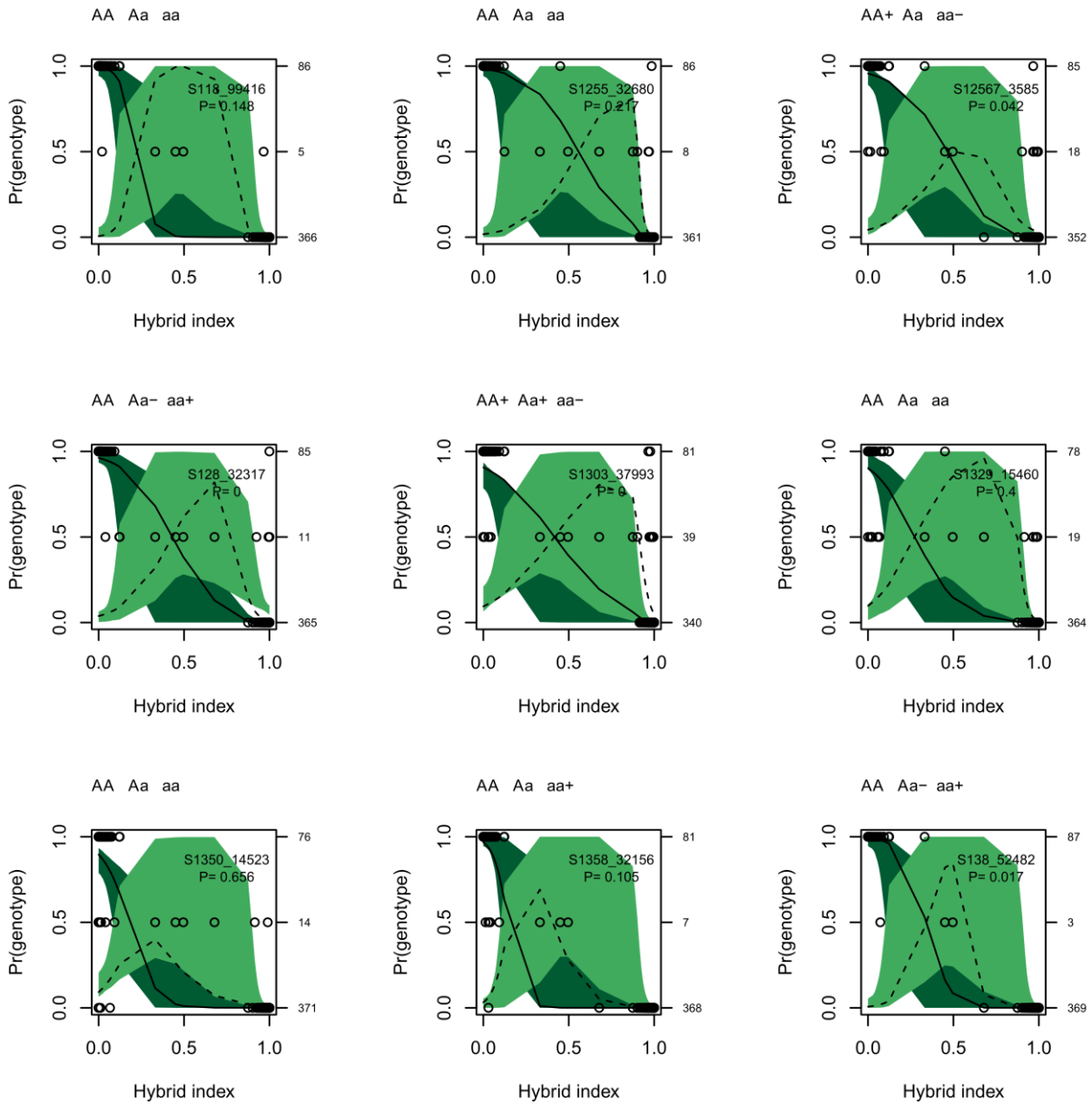
Wu, C.-I. 2001. The genic view of the process of speciation. Journal of Evolutionary Biology, 14: 851-865.

Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C., and B.S. Weir. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics, 28: 3326-3328.

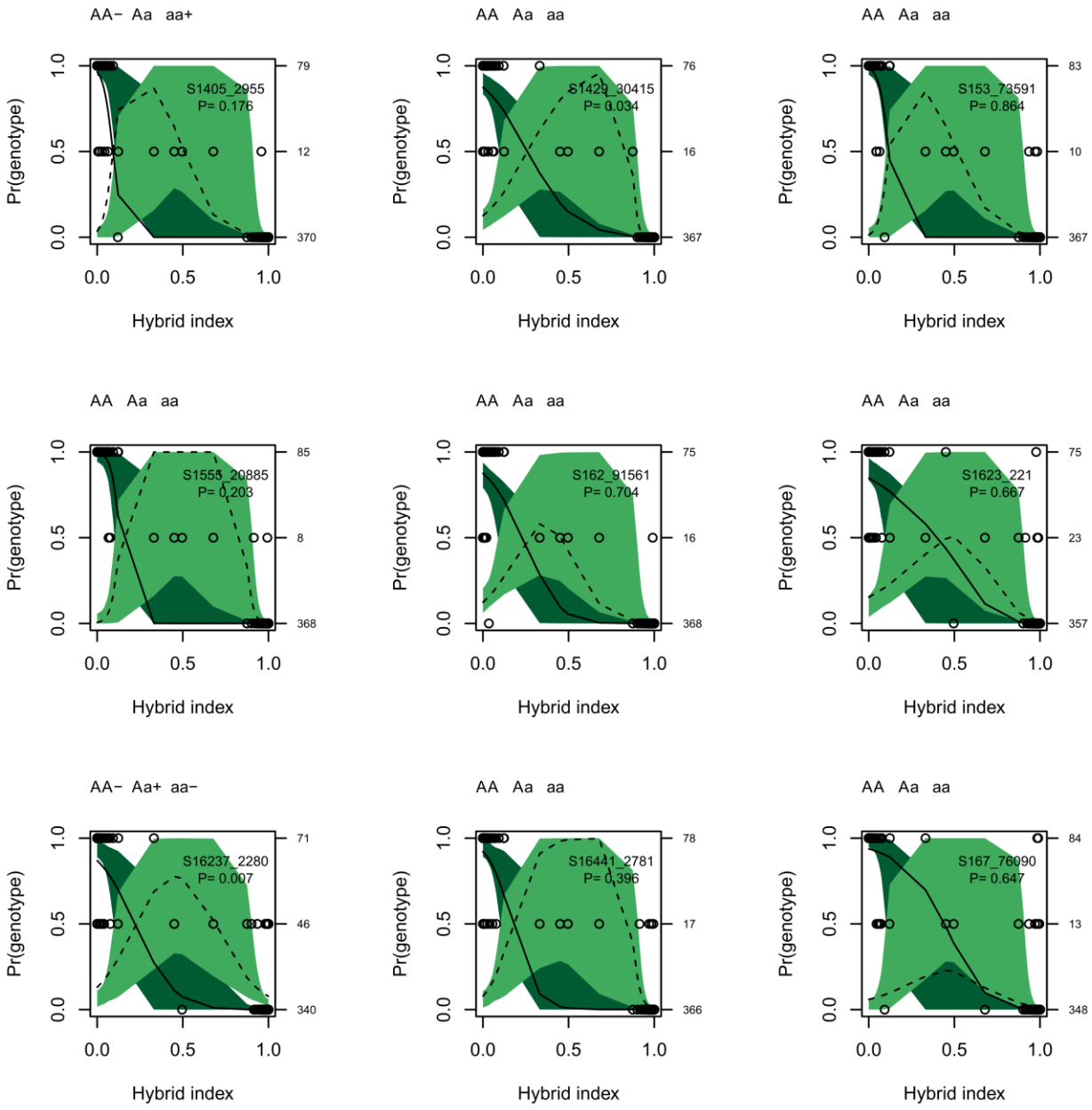
Appendix 5-1: Genomic clines plotted as probability of homozygous and heterozygous genotypes against hybrid indices between *C. fumiferana* (0, A) and *C. occidentalis* (1, a), excesses (+) and deficits (-) of genotypes relative to neutral introgression are noted above with significance of deviation noted inside the plot with 95% confidence intervals for homozgyotes (dark green) and heterozygotes (light green), right-hand vertical axis indicates sample size.



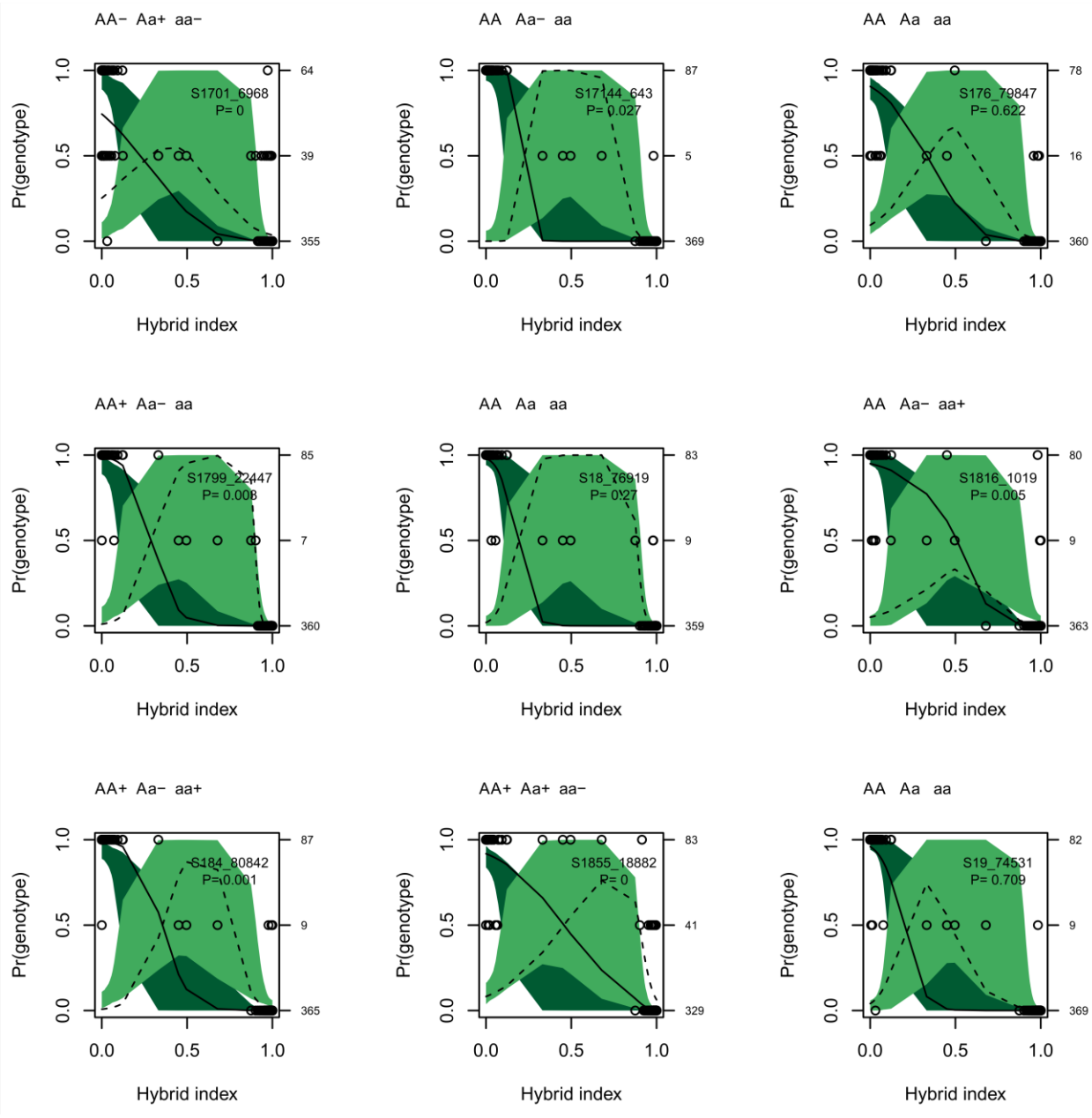
Appendix 5-1: (continued)



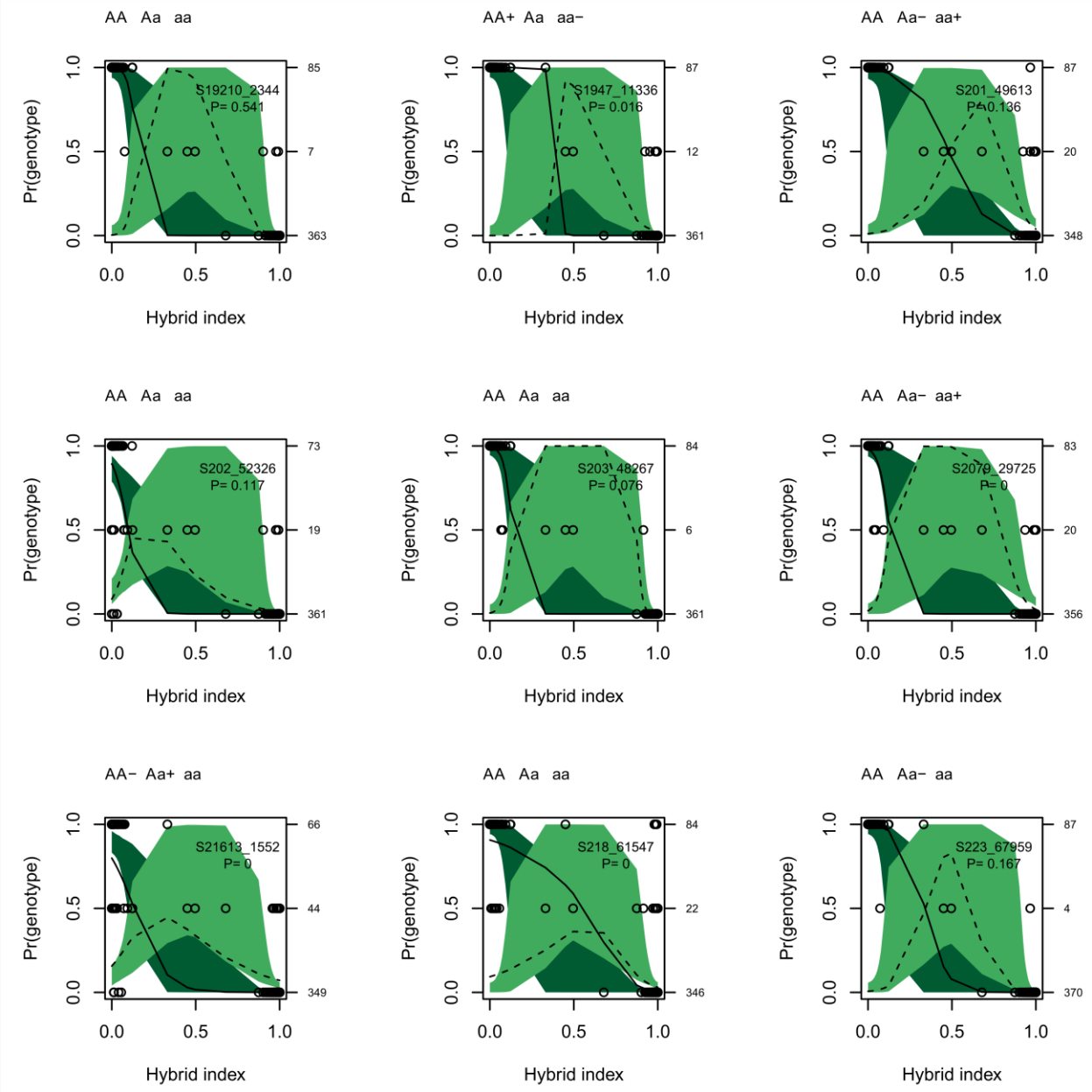
Appendix 5-1: (continued)



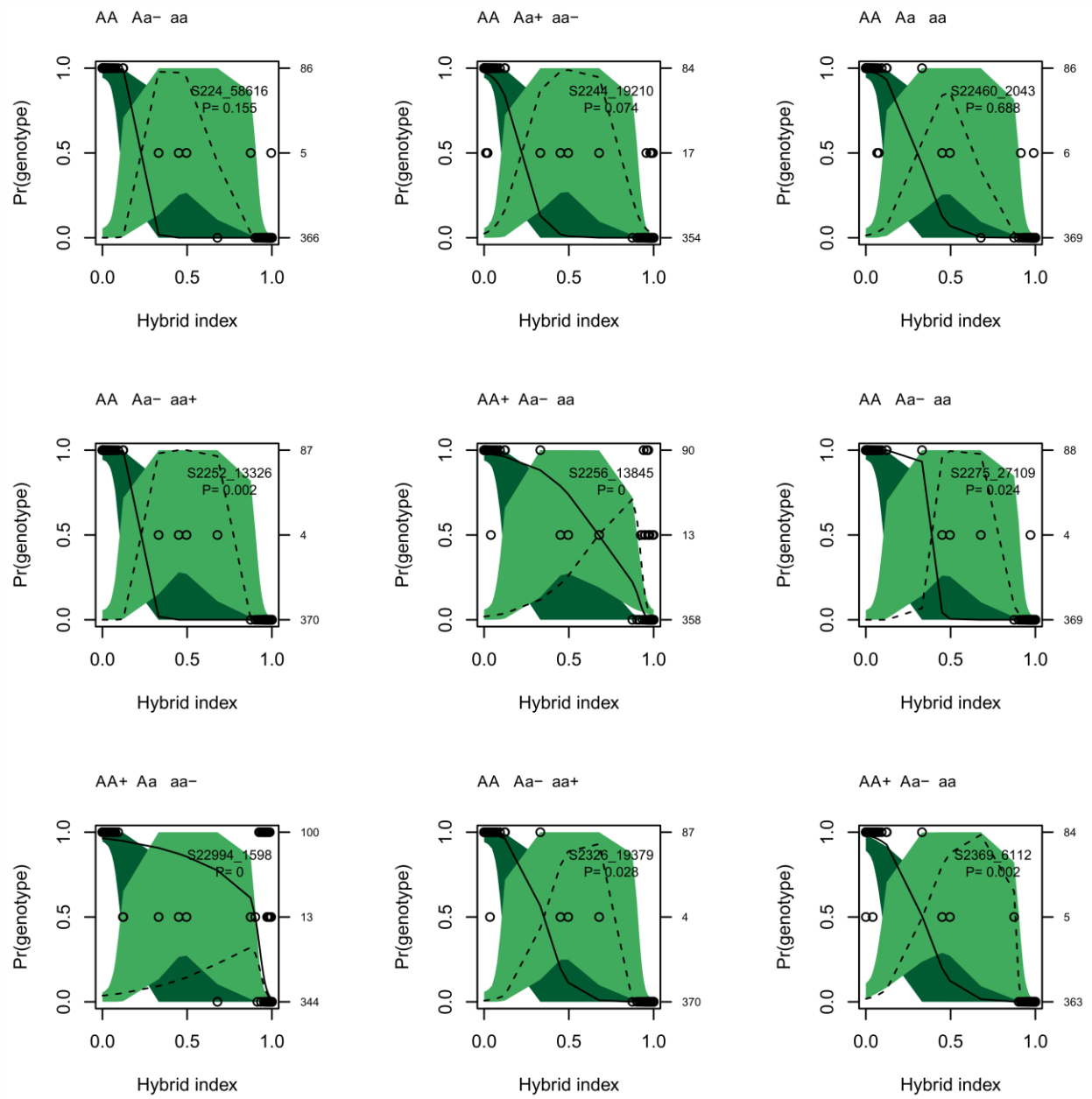
Appendix 5-1: (continued)



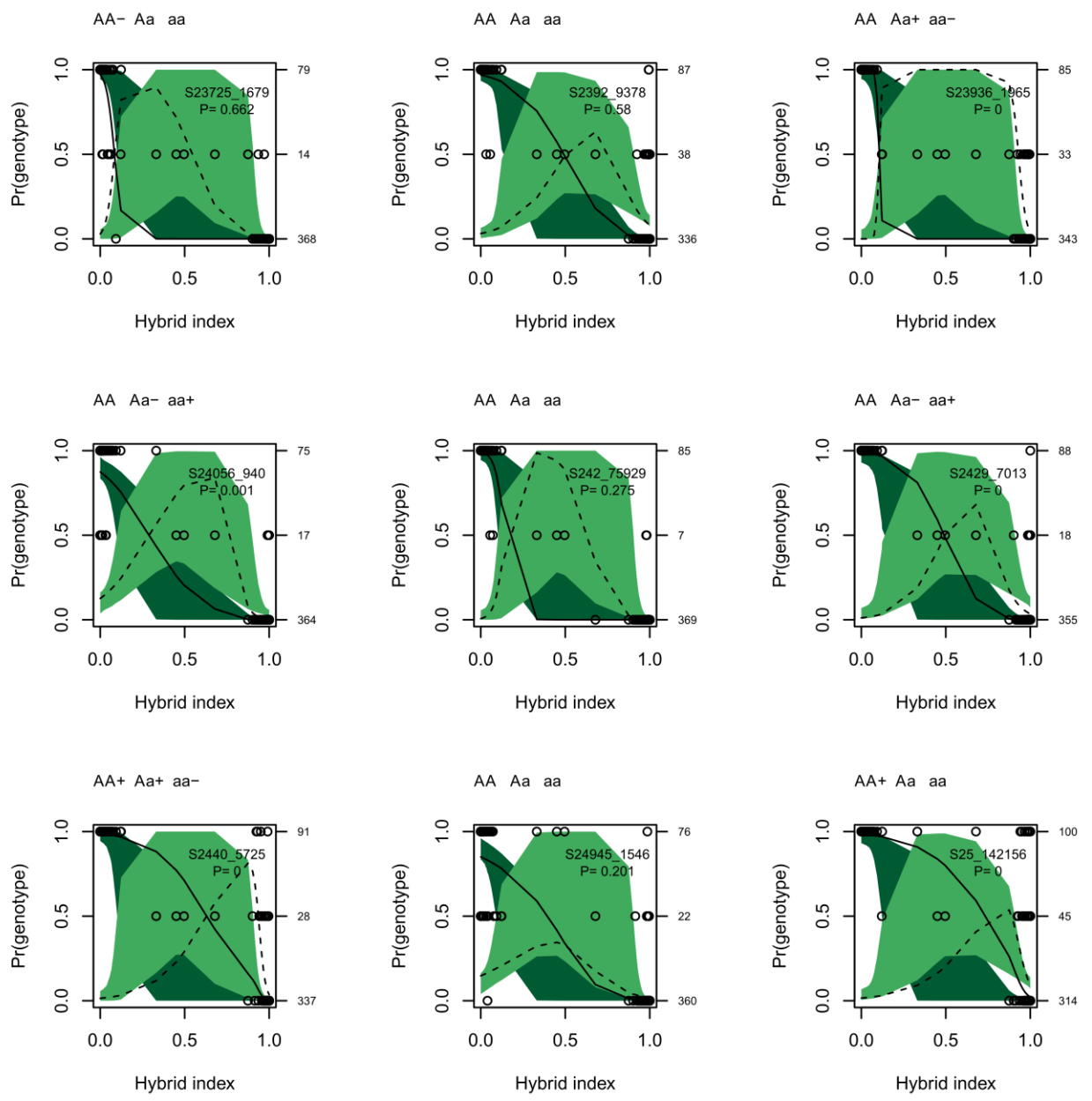
Appendix 5-1: (continued)



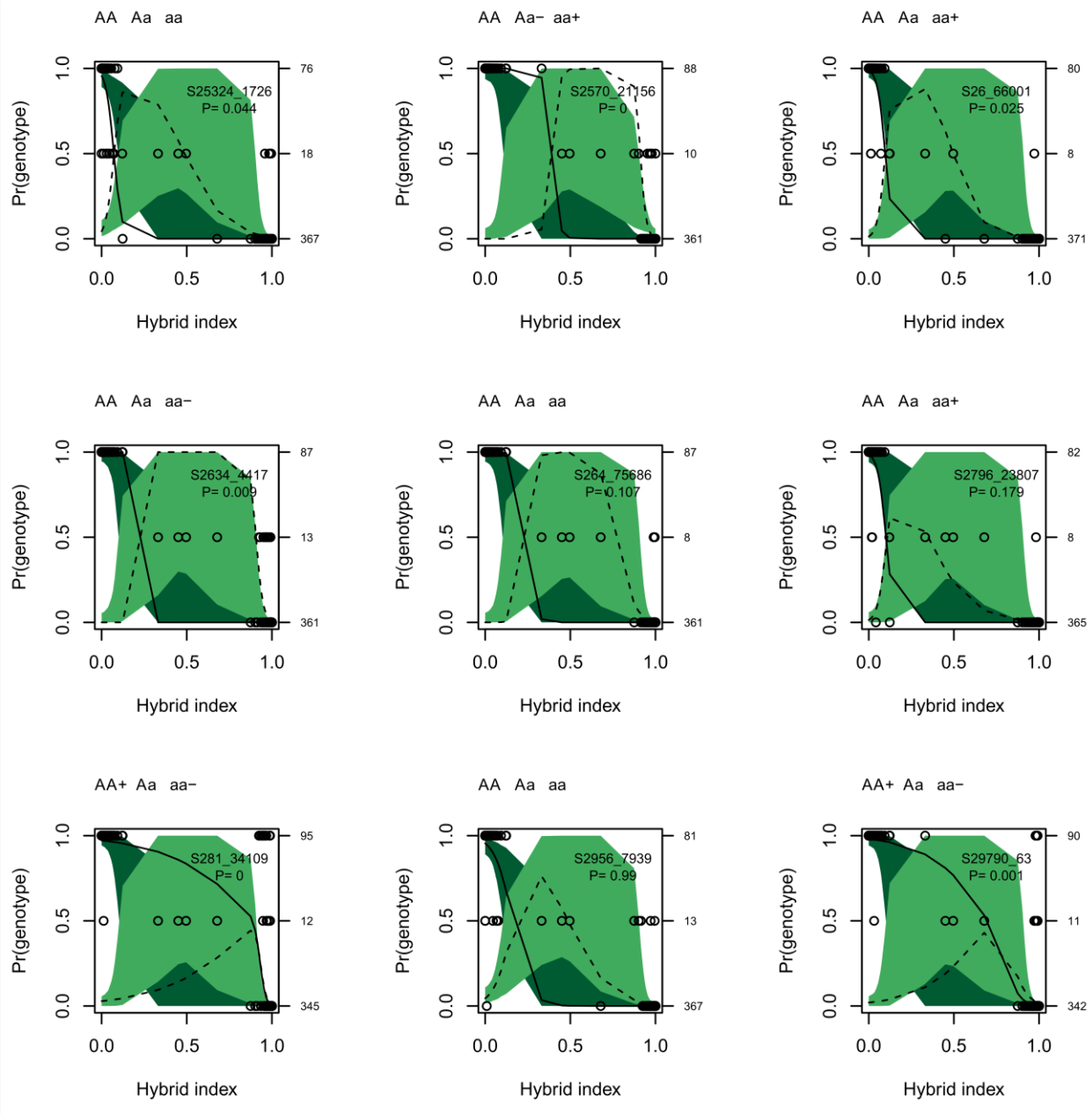
Appendix 5-1: (continued)



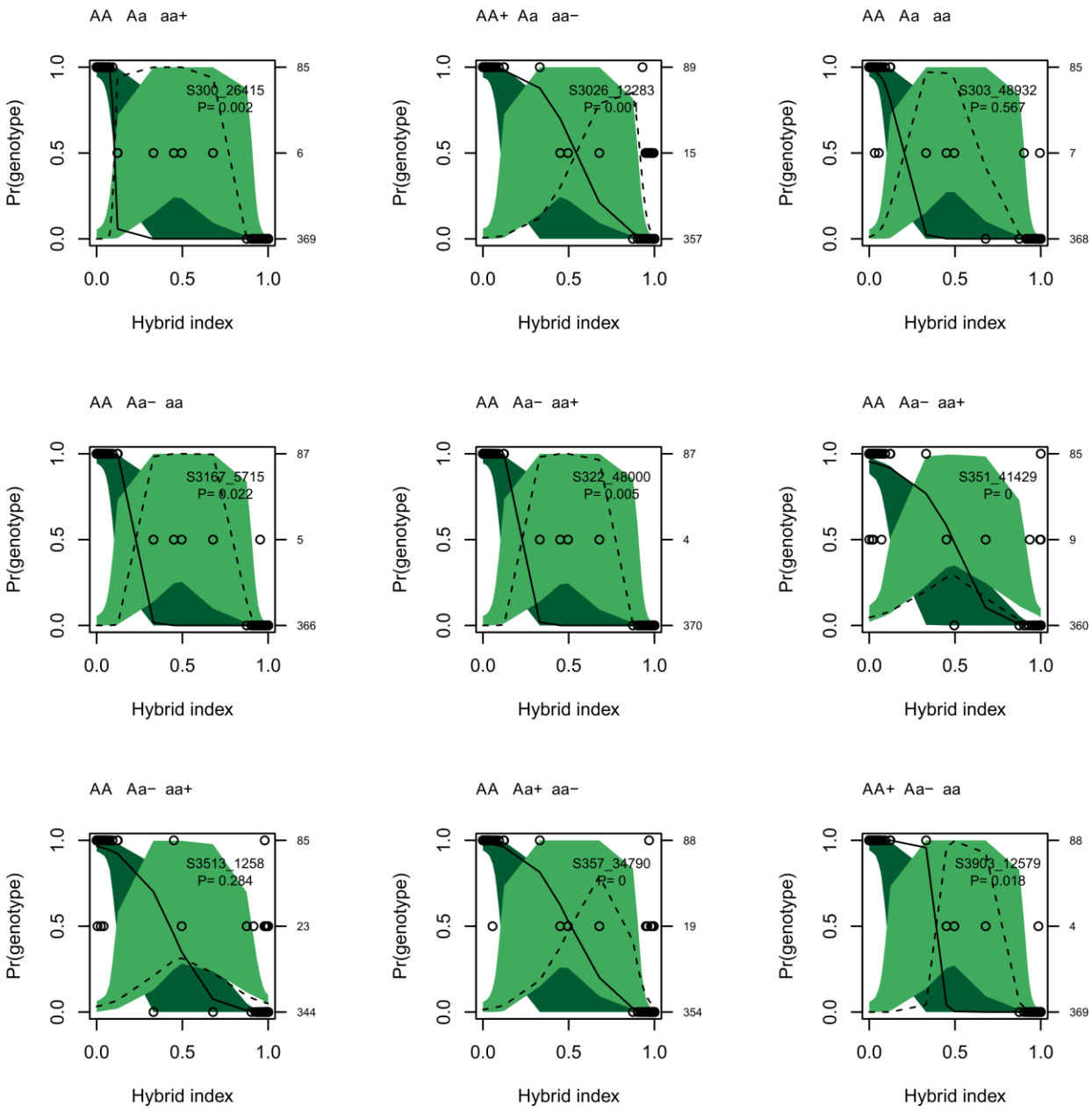
Appendix 5-1: (continued)



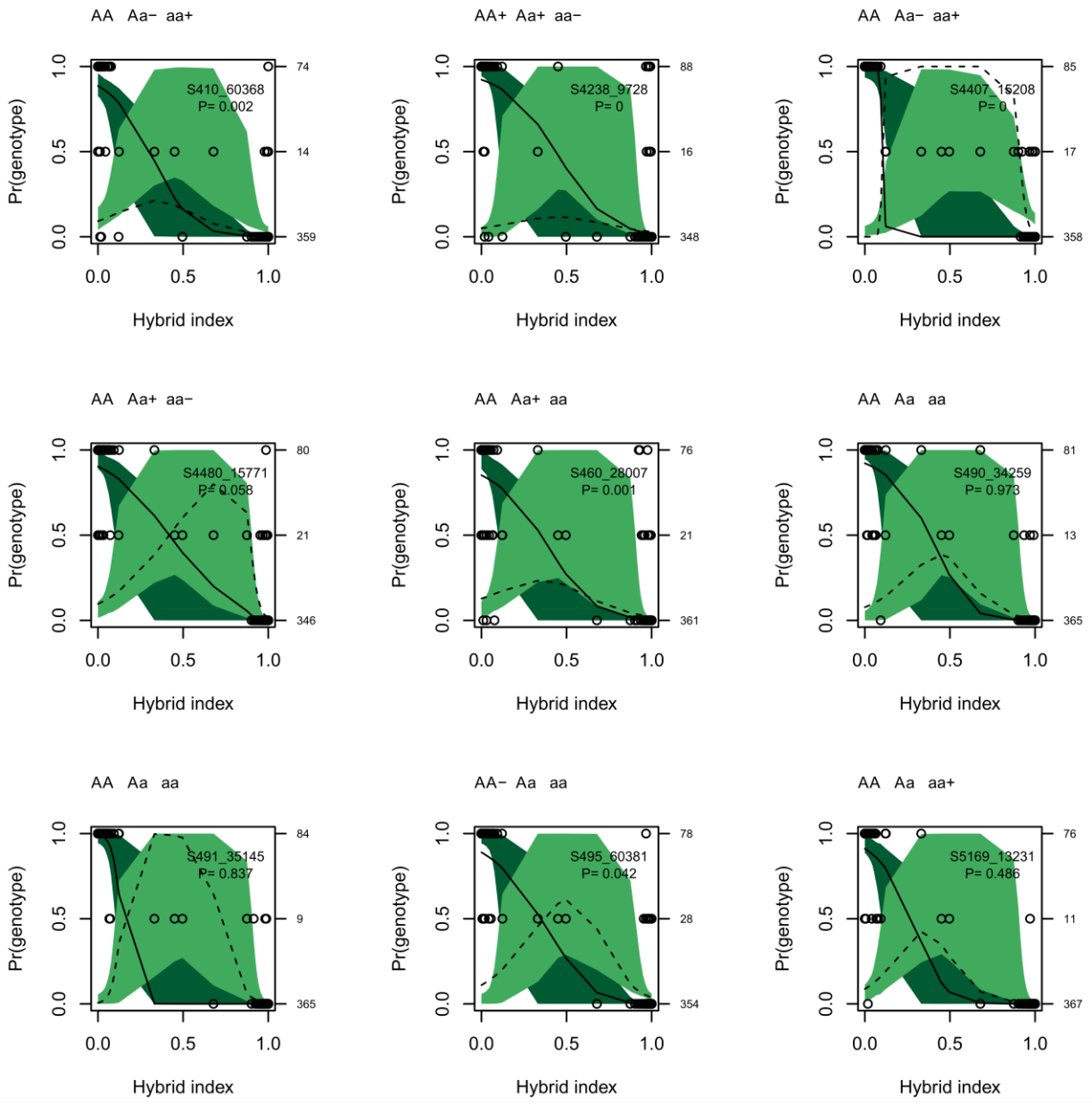
Appendix 5-1: (continued)



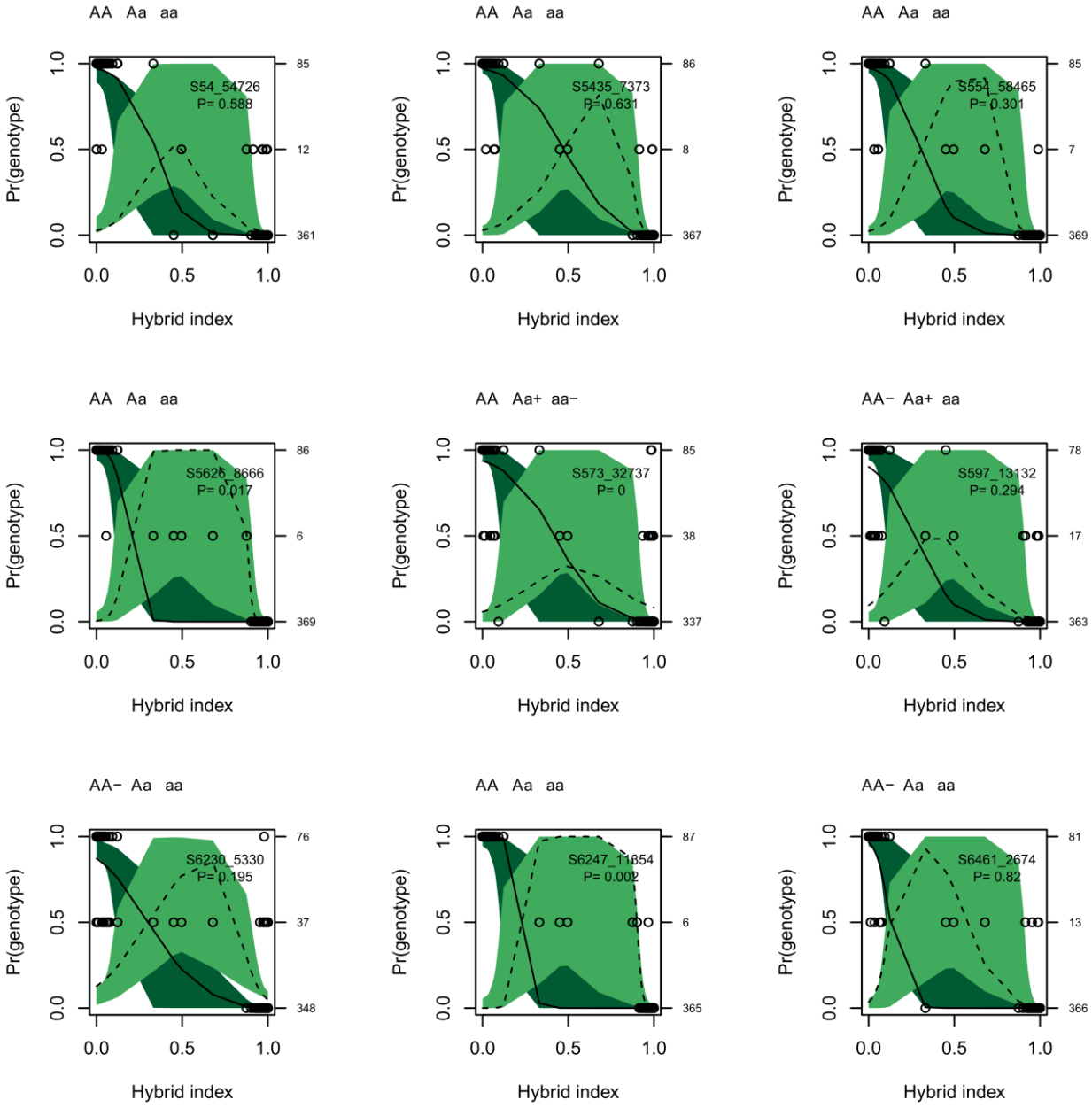
Appendix 5-1: (continued)



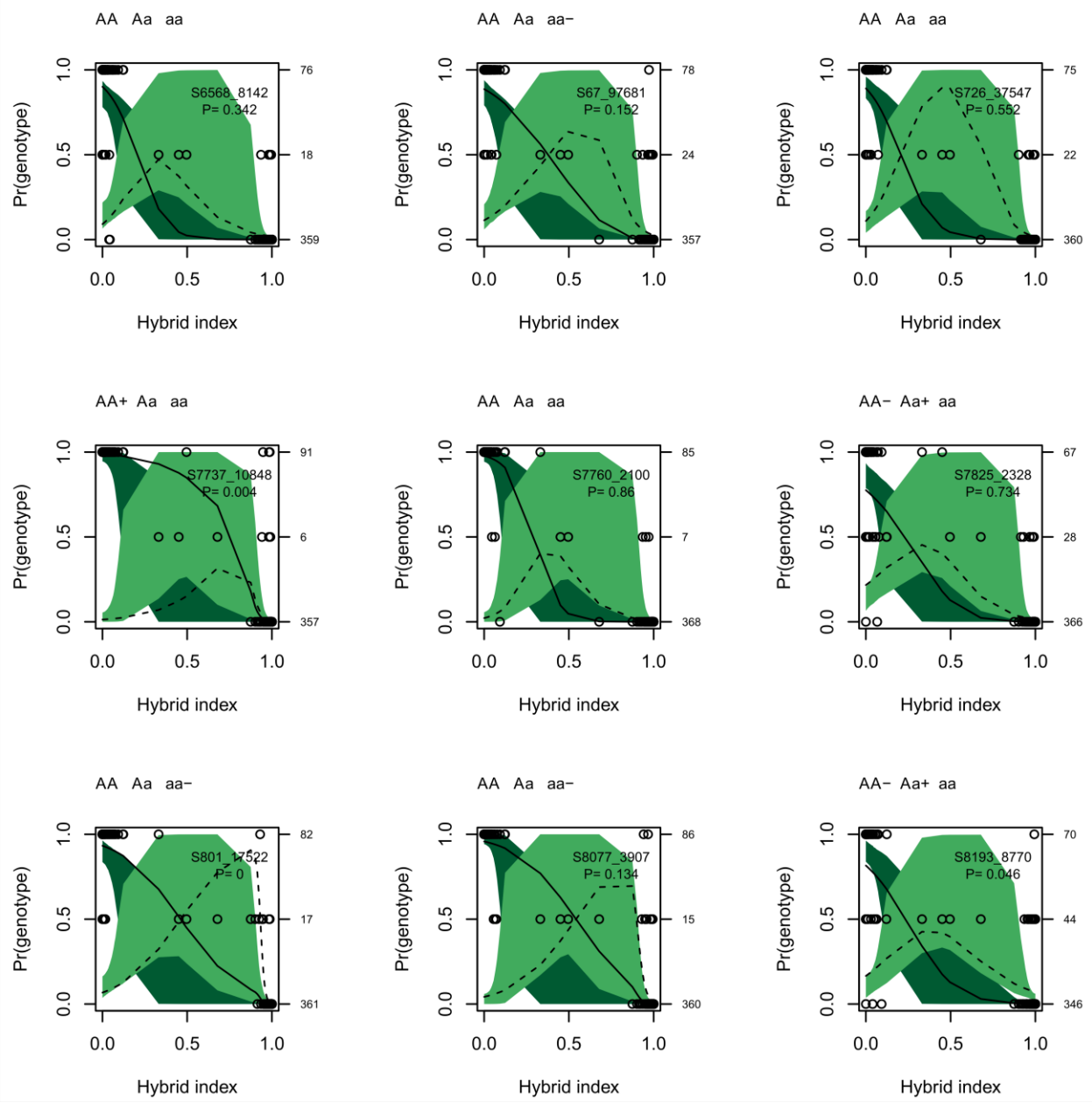
Appendix 5-1: (continued)



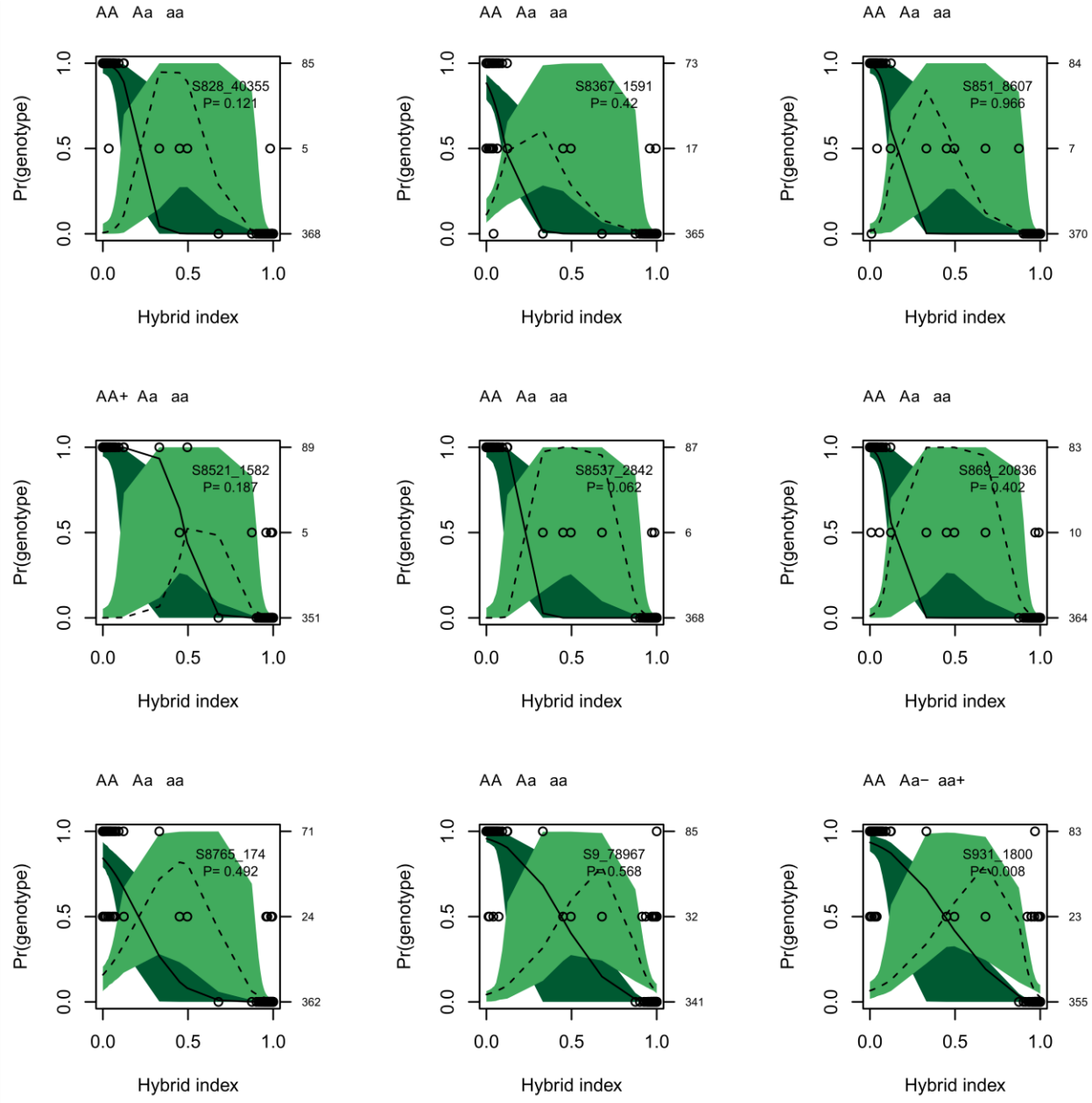
Appendix 5-1: (continued)



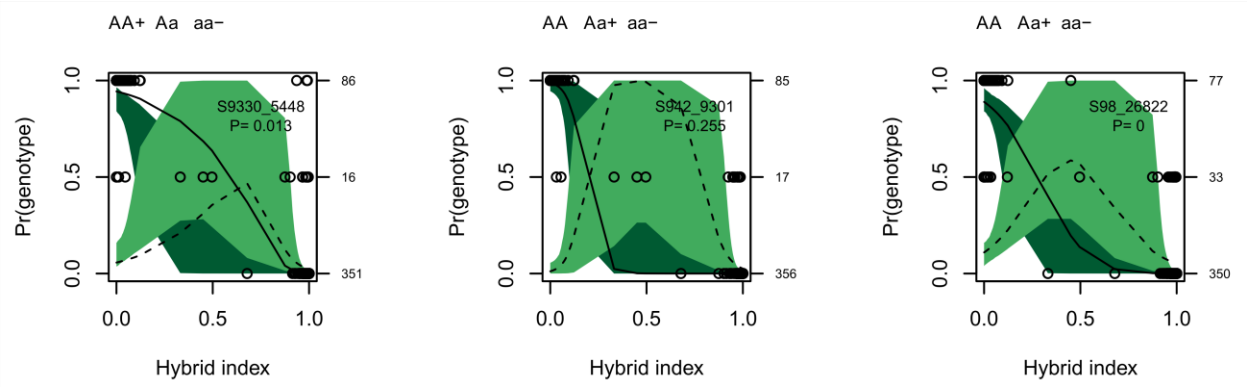
Appendix 5-1: (continued)



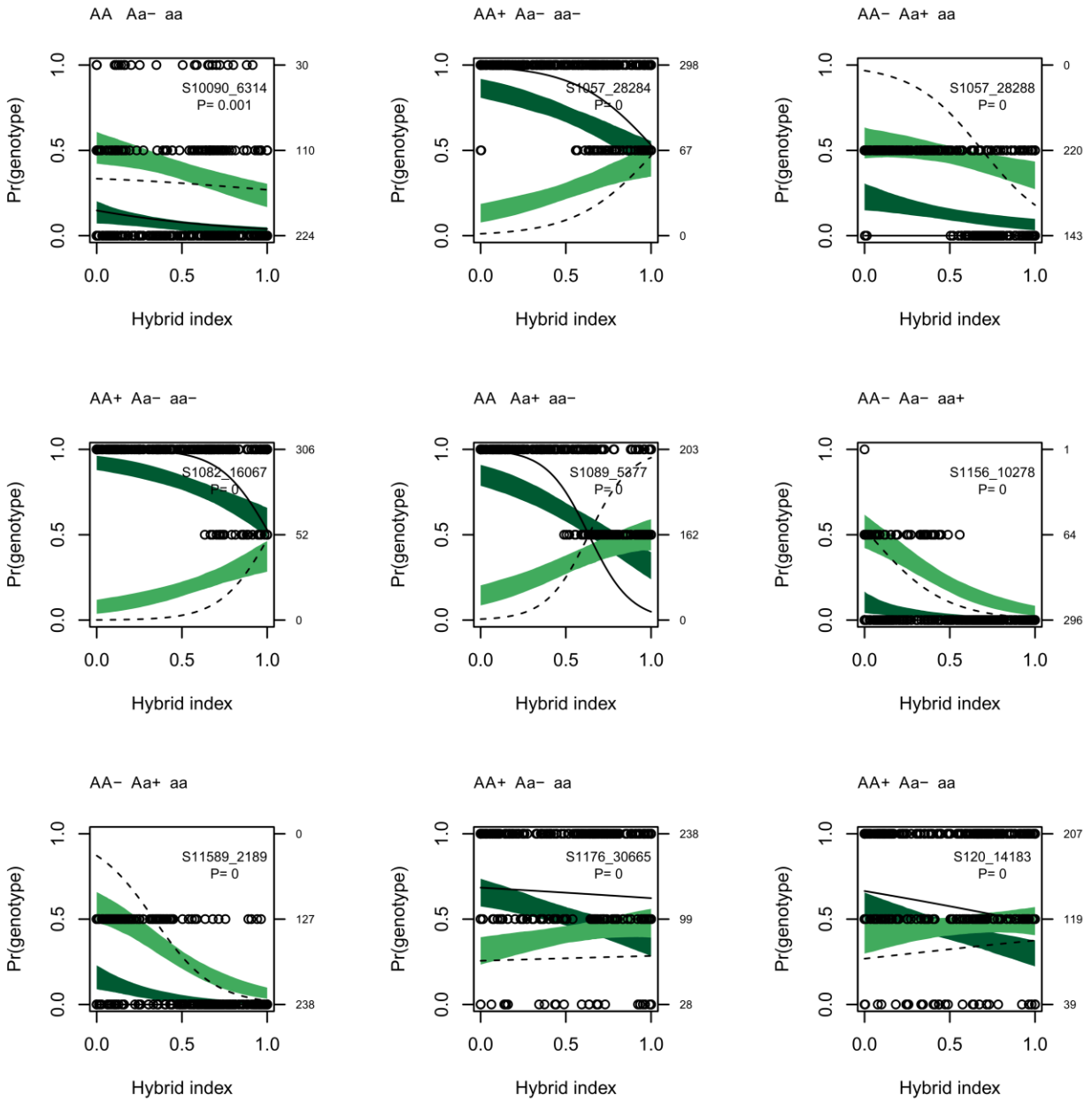
Appendix 5-1: (continued)



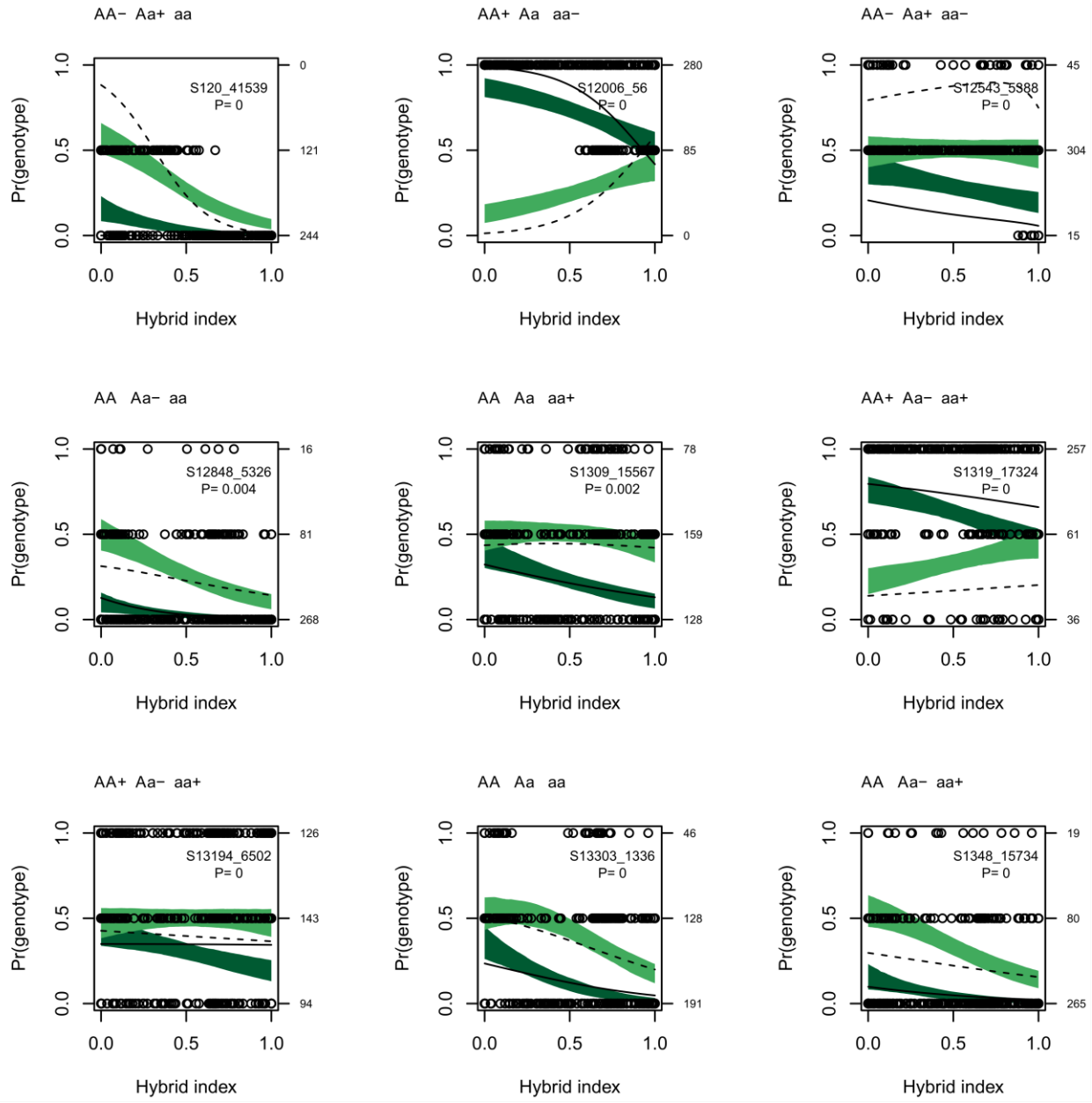
Appendix 5-1: (continued)



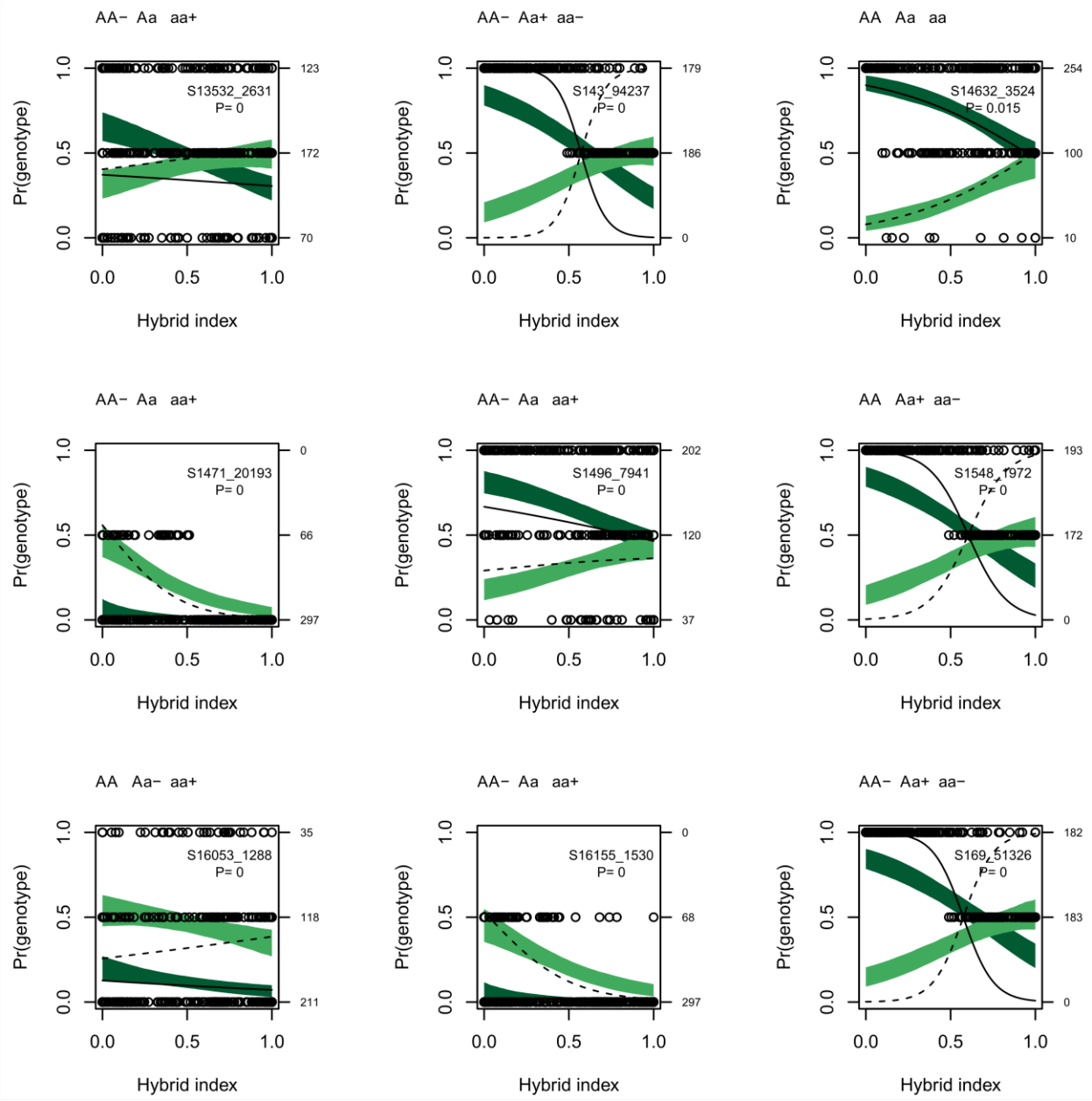
Appendix 5-2: Genomic clines plotted as probability of homozygous and heterozygous genotypes against hybrid indices between *C. biennis* (0, A) and *C. occidentalis* (1, a), excesses (+) and deficits (-) of genotypes relative to neutral introgression are noted above with significance of deviation noted inside the plot with 95% confidence intervals for homozgyotes (dark green) and heterozygotes (light green), right-hand vertical axis indicates sample size.



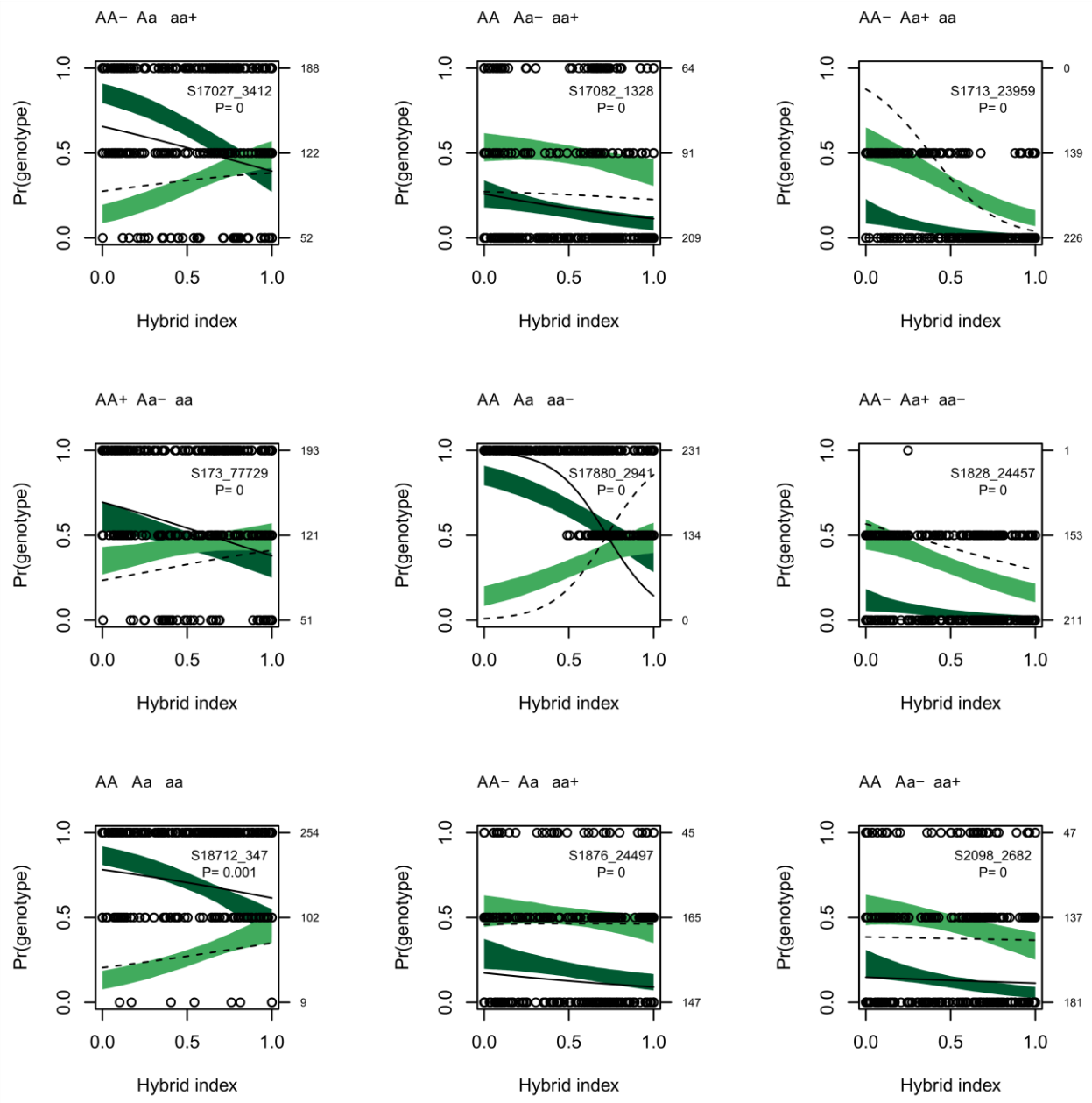
Appendix 5-2: (continued)



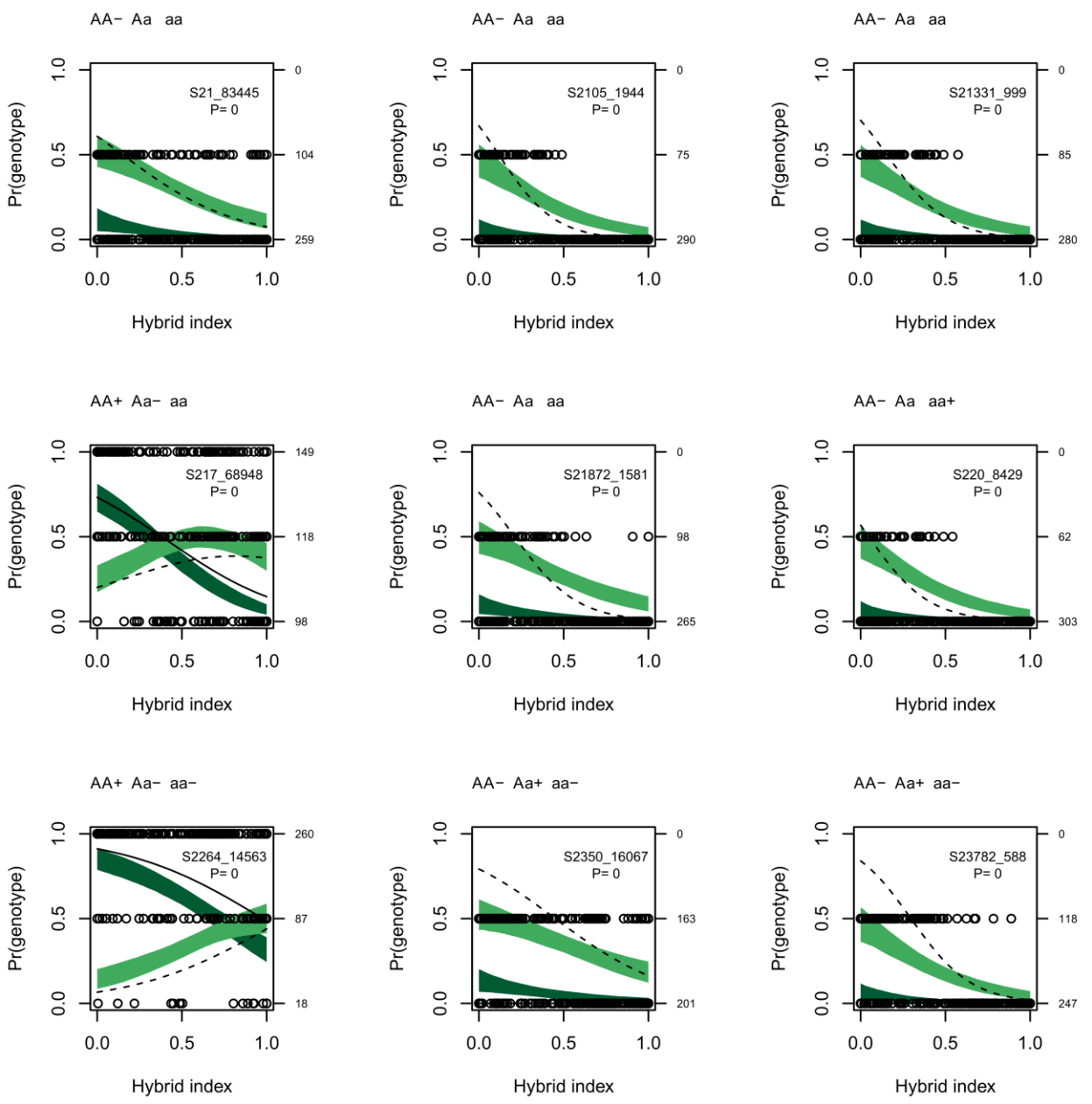
Appendix 5-2: (continued)



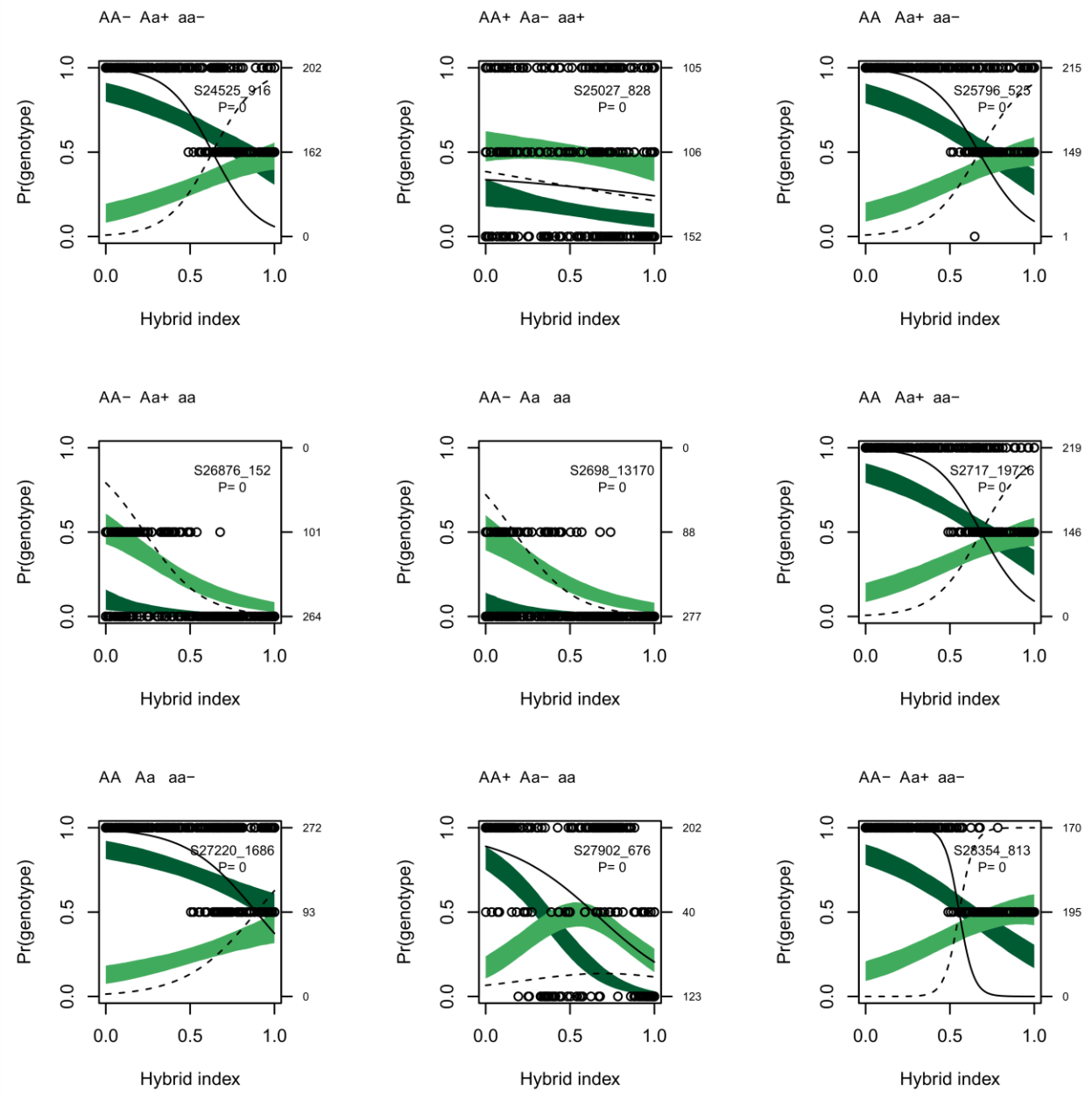
Appendix 5-2: (continued)



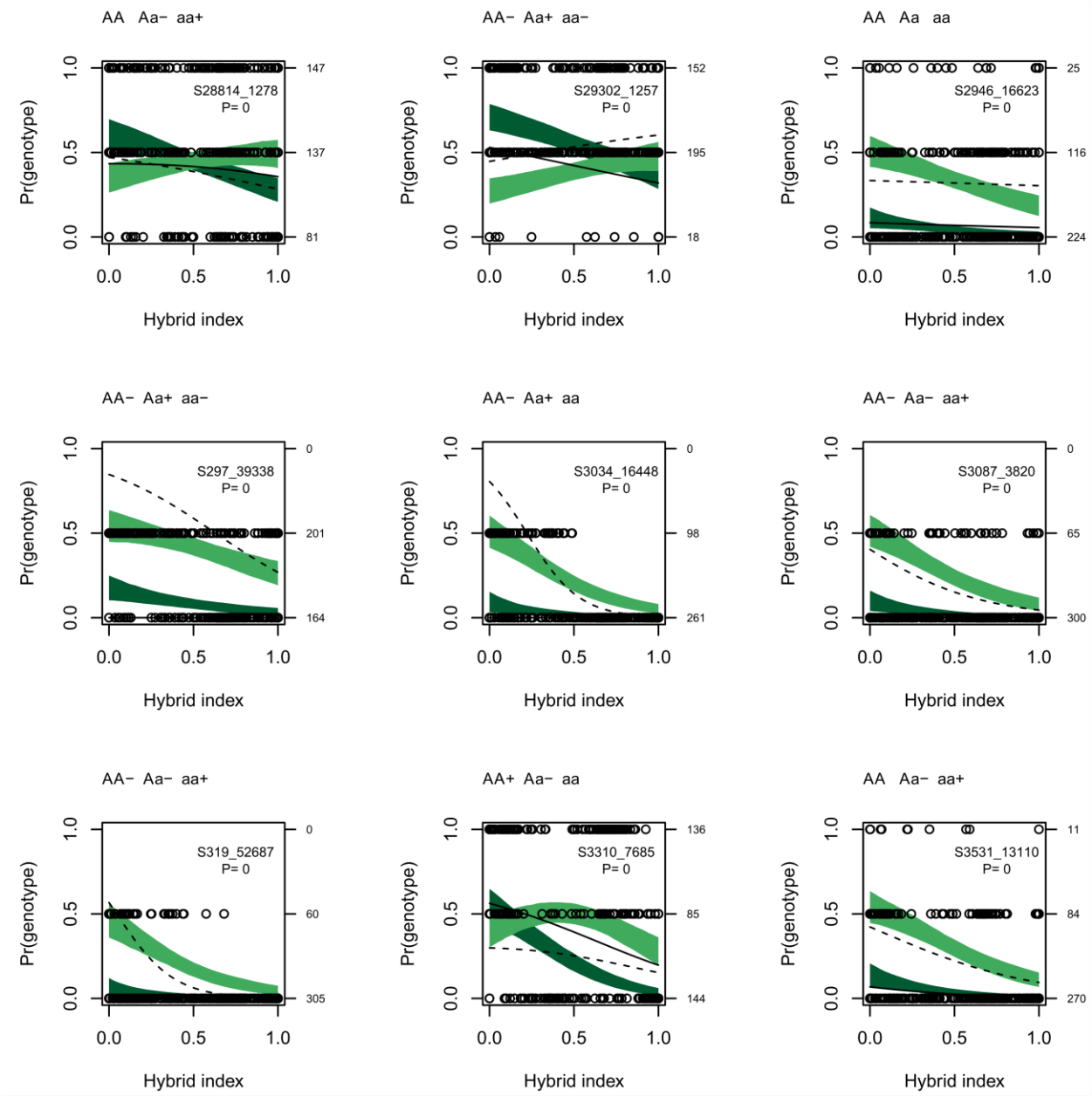
Appendix 5-2: (continued)



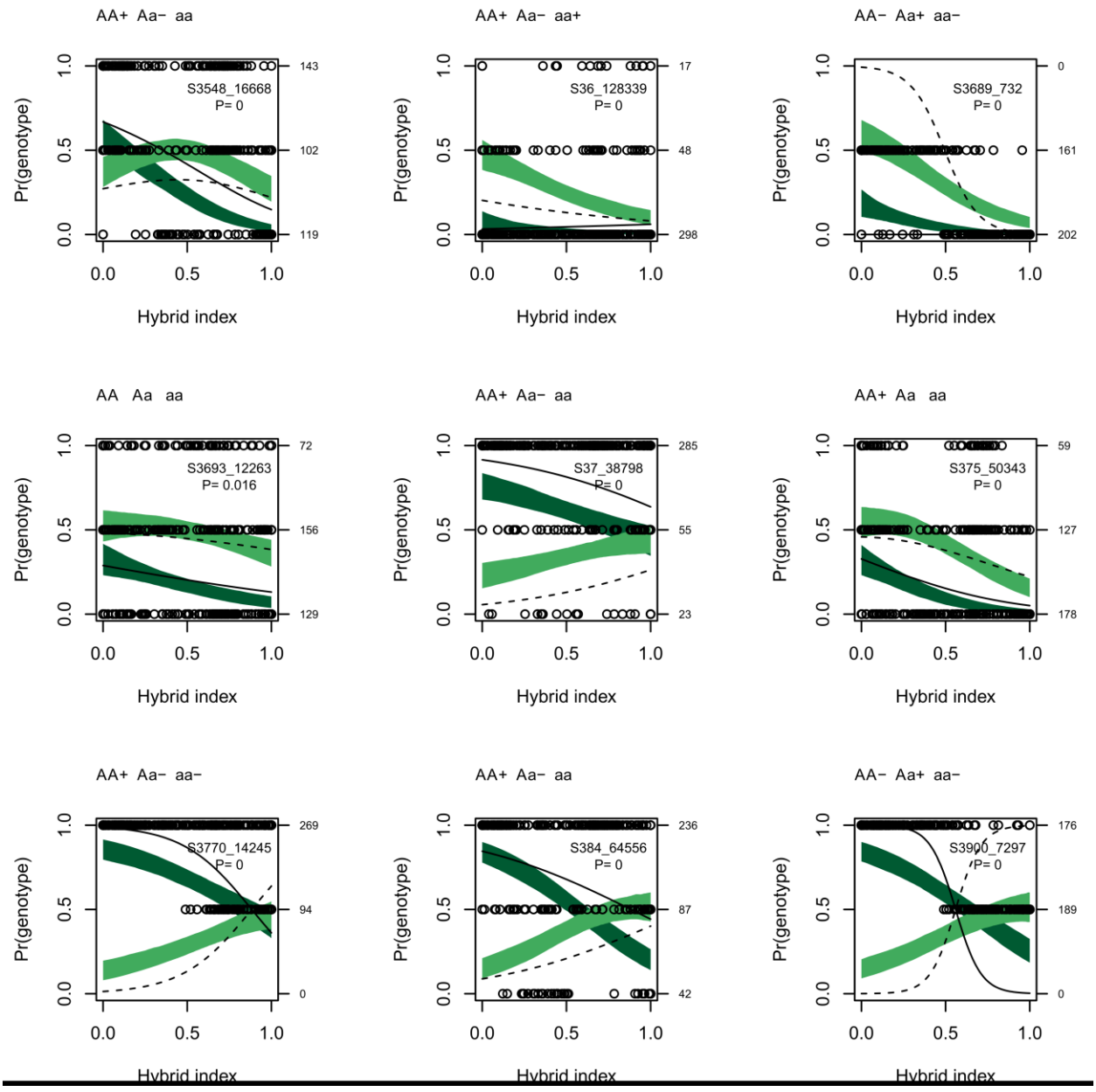
Appendix 5-2: (continued)



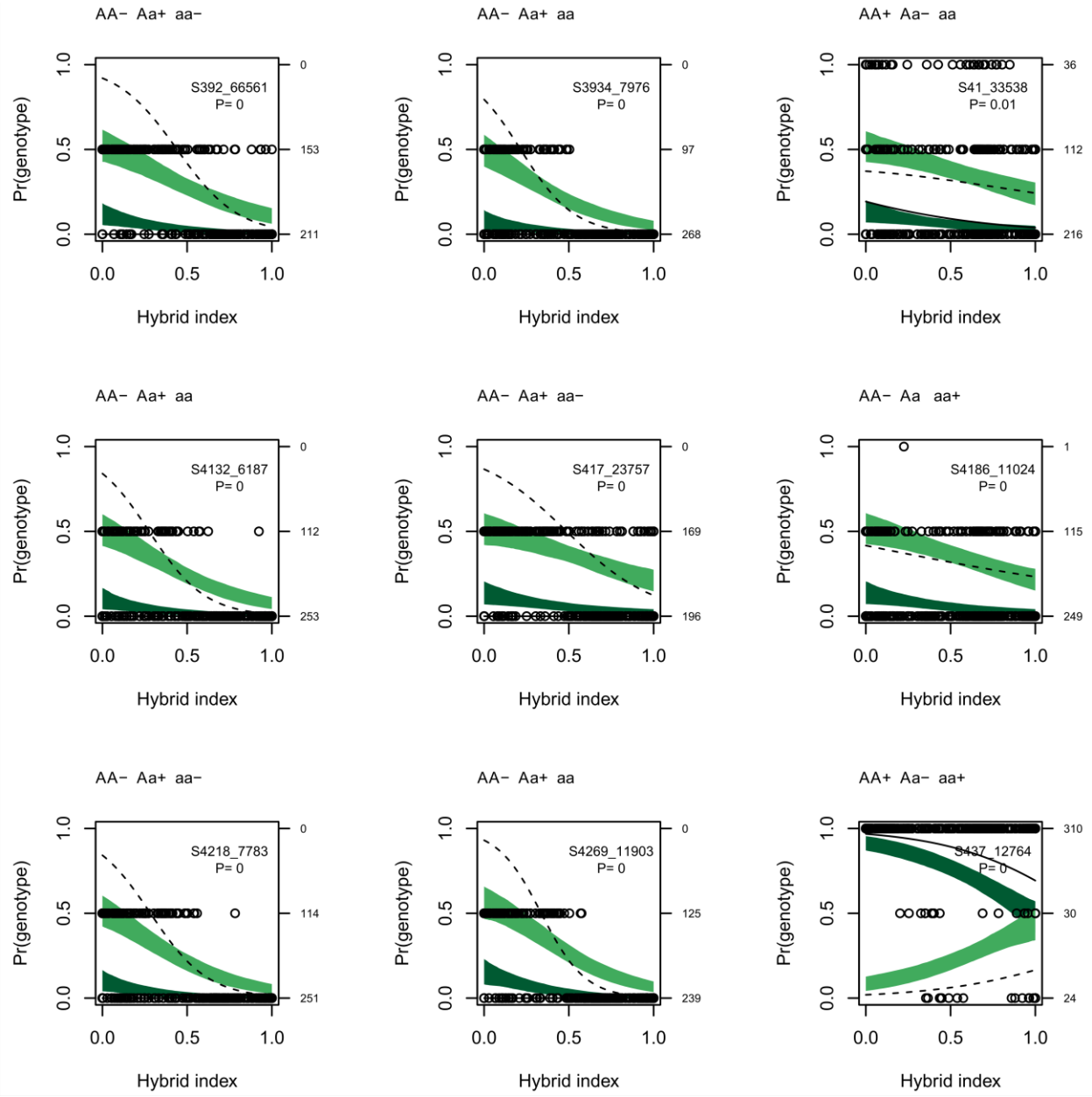
Appendix 5-2: (continued)



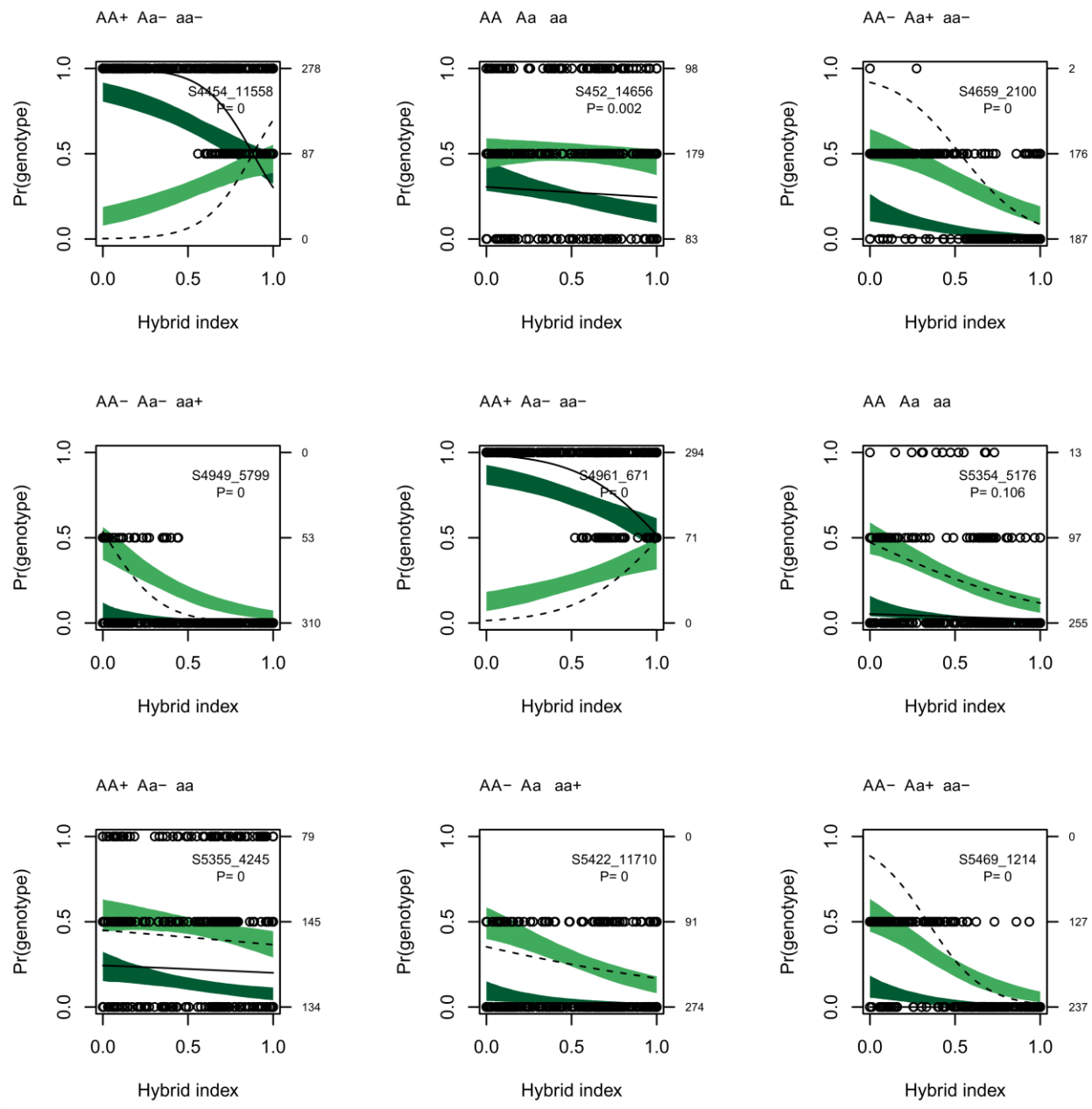
Appendix 5-2: (continued)



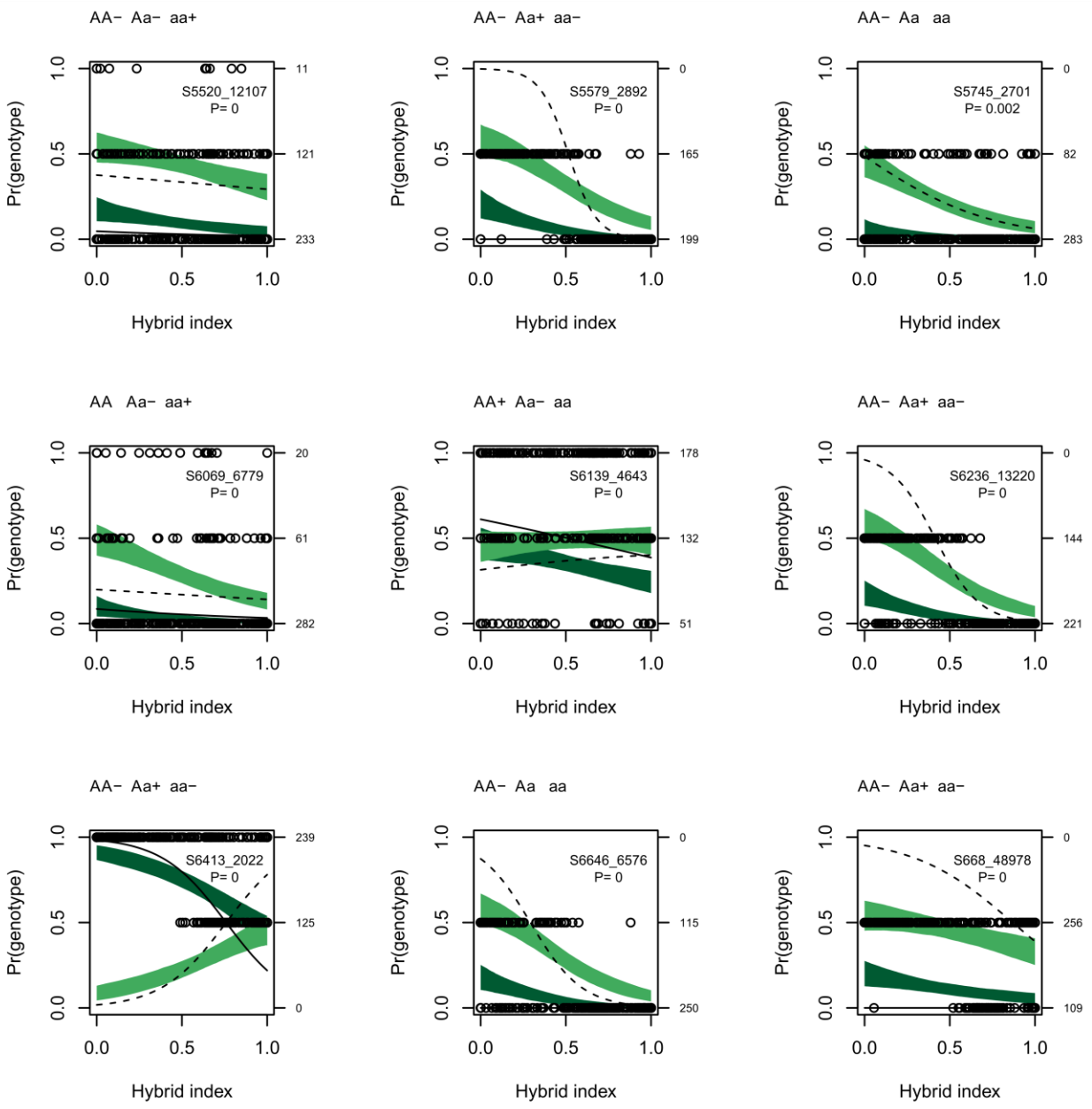
Appendix 5-2: (continued)



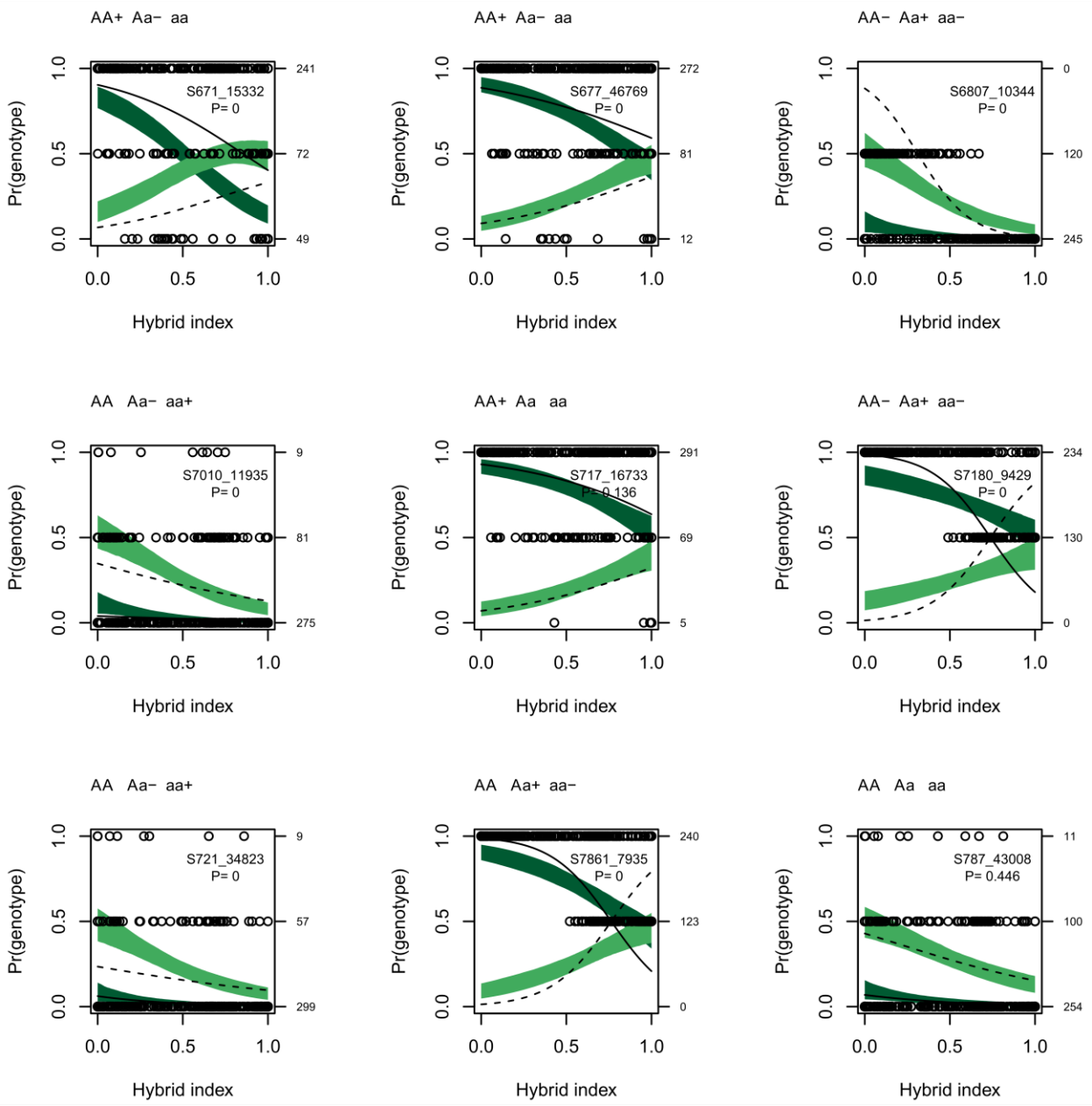
Appendix 5-2: (continued)



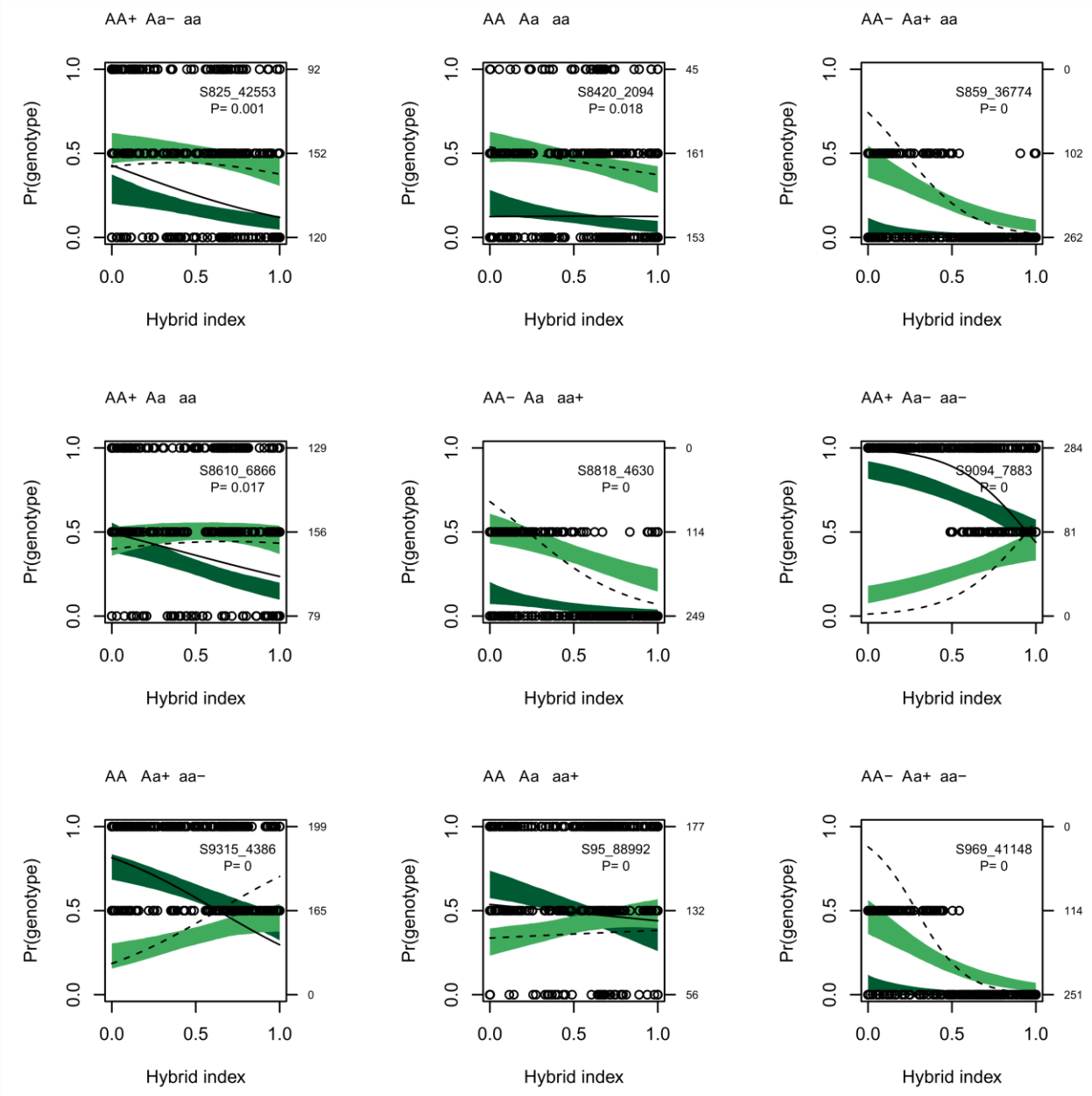
Appendix 5-2: (continued)



Appendix 5-2: (continued)



Appendix 5-2: (continued)



Appendix 5-3: BayeScan genome scan test statistics for significant Fst outliers. Prob is the posterior probability for selection, $\log_{10}$(PO) is the logarithm of posterior odds for selection, qvalue is the significance corrected for a false discovery rate of 5%, alpha is the coefficient of selection (positive indicates diversifying selection, negative indicates balancing selection), Fst is the genetic differentiation averaged over populations.

	C. fumiferana vs. C. biennis					*C. fumiferana vs. C. occidentalis*					*C. occidentalis vs. C. biennis*				
Locus	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst
S1_781981	0.97	1.54	0.02	2.07	0.71	1.00	2.62	0.00	2.22	0.72	0.00	-3.22	0.99	0.00	0.03
S1083_32522	0.97	1.58	0.02	2.05	0.70	1.00	2.58	0.00	2.21	0.72	0.00	-3.10	0.99	0.00	0.03
S1093_15778	0.98	1.65	0.02	2.06	0.70	1.00	2.55	0.00	2.20	0.72	0.00	-3.10	0.99	0.00	0.03
S11686_2436	0.98	1.61	0.02	2.07	0.71	1.00	2.58	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S118_99416	0.97	1.58	0.02	2.07	0.71	1.00	2.58	0.00	2.20	0.72	0.00	-3.70	1.00	0.00	0.03
S1255_32680	0.97	1.49	0.02	2.03	0.70	1.00	2.42	0.00	2.19	0.72	0.00	-3.70	1.00	0.00	0.03
S128_32317	0.98	1.63	0.02	2.06	0.71	0.78	0.56	0.02	1.49	0.58	0.00	-1000.00	1.00	0.00	0.03
S1358_32156	0.97	1.47	0.03	2.04	0.70	1.00	2.32	0.00	2.20	0.72	0.00	-1000.00	1.00	0.00	0.03
S138_52482	0.97	1.58	0.02	2.06	0.71	1.00	2.55	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S1405_2955	0.96	1.44	0.03	2.06	0.70	1.00	2.52	0.00	2.22	0.72	0.00	-1000.00	1.00	0.00	0.03
S153_73591	0.97	1.57	0.02	2.04	0.70	1.00	2.52	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S1555_20885	0.98	1.64	0.02	2.06	0.71	1.00	2.42	0.00	2.21	0.72	0.00	-3.10	0.99	0.00	0.03
S167_76090	0.98	1.66	0.02	2.07	0.71	1.00	2.66	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S17144_643	0.98	1.62	0.02	2.07	0.71	1.00	2.42	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S18_76919	0.95	1.27	0.03	1.98	0.69	1.00	2.55	0.00	2.21	0.72	0.00	-3.40	1.00	0.00	0.03
S1816_1019	0.96	1.35	0.03	2.03	0.70	0.90	0.94	0.01	1.74	0.63	0.00	-3.10	0.99	0.00	0.03
S19_74531	0.97	1.50	0.02	2.03	0.70	1.00	2.47	0.00	2.22	0.72	0.00	-3.22	0.99	0.00	0.03
S19210_2344	0.98	1.66	0.02	2.07	0.71	1.00	2.66	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S1947_11336	0.97	1.49	0.02	2.04	0.70	0.99	2.25	0.00	2.20	0.72	0.00	-3.70	1.00	0.00	0.03
S201_49613	0.96	1.38	0.03	2.02	0.70	0.79	0.58	0.01	1.49	0.58	0.00	-3.40	1.00	0.00	0.03
S203_48267	0.97	1.45	0.03	2.03	0.70	0.99	2.09	0.00	2.17	0.71	0.00	-3.70	1.00	0.00	0.03
S2079_29725	0.98	1.61	0.02	2.06	0.71	0.84	0.73	0.01	1.59	0.60	0.00	-3.70	1.00	0.00	0.03
S218_61547	0.01	-2.06	0.36	0.01	0.27	0.92	1.07	0.01	1.78	0.64	0.00	-3.70	1.00	0.00	0.03
S223_67959	0.97	1.56	0.02	2.06	0.71	1.00	2.49	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03

Appendix 5-3: (continued)

	C. fumiferana vs. C. biennis					*C. fumiferana vs. C. occidentalis*					*C. occidentalis vs. C. biennis*				
Locus	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst
S224_58616	0.98	1.60	0.02	2.07	0.71	1.00	2.52	0.00	2.21	0.72	0.00	-3.10	0.99	0.00	0.03
S2244_19210	0.98	1.64	0.02	2.05	0.70	1.00	2.74	0.00	2.21	0.72	0.00	-3.40	1.00	0.00	0.03
S22460_2043	0.98	1.64	0.02	2.07	0.71	1.00	2.55	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S2252_13326	0.98	1.66	0.02	2.06	0.70	1.00	2.85	0.00	2.20	0.72	0.00	-3.40	1.00	0.00	0.03
S2256_13845	0.97	1.53	0.02	2.05	0.70	0.94	1.20	0.00	1.82	0.65	0.00	-3.40	1.00	0.00	0.03
S2275_27109	0.98	1.61	0.02	2.07	0.71	1.00	2.44	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S22994_1598	0.97	1.56	0.02	2.03	0.70	1.00	2.42	0.00	2.19	0.72	0.00	-3.22	0.99	0.00	0.03
S2326_19379	0.97	1.59	0.02	2.07	0.71	1.00	2.40	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S23725_1679	0.97	1.57	0.02	2.08	0.71	1.00	2.47	0.00	2.22	0.72	0.00	-3.70	1.00	0.00	0.03
S23936_1965	0.54	0.07	0.06	1.00	0.49	1.00	2.62	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S242_75929	0.97	1.49	0.03	2.04	0.70	1.00	2.42	0.00	2.21	0.72	0.00	-3.70	1.00	0.00	0.03
S2429_7013	0.98	1.60	0.02	2.04	0.70	0.00	-1000.00	0.96	0.00	0.27	0.00	-3.22	0.99	0.00	0.03
S2440_5725	0.98	1.67	0.02	2.08	0.71	1.00	2.58	0.00	2.20	0.72	0.00	-3.22	0.99	0.00	0.03
S2570_21156	0.98	1.64	0.02	2.07	0.71	0.94	1.16	0.00	1.82	0.65	0.00	-1000.00	1.00	0.00	0.03
S26_66001	0.97	1.59	0.02	2.07	0.71	1.00	2.66	0.00	2.22	0.72	0.00	-3.70	1.00	0.00	0.03
S2634_4417	0.97	1.55	0.02	2.05	0.70	1.00	2.62	0.00	2.23	0.73	0.00	-3.10	0.99	0.00	0.03
S264_75686	0.97	1.55	0.02	2.05	0.70	1.00	2.37	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S27902_676	0.55	0.08	0.05	1.01	0.49	0.00	-3.22	0.92	0.00	0.27	1.00	1000.00	0.00	3.01	0.36
S2796_23807	0.98	1.70	0.02	2.06	0.71	1.00	2.58	0.00	2.20	0.72	0.00	-3.70	1.00	0.00	0.03
S281_34109	0.96	1.35	0.03	2.01	0.69	1.00	2.49	0.00	2.21	0.72	0.00	-3.70	1.00	0.00	0.03
S29790_63	0.92	1.05	0.03	1.90	0.67	1.00	2.47	0.00	2.18	0.72	0.00	-3.70	1.00	0.00	0.03
S300_26415	0.98	1.67	0.02	2.08	0.71	1.00	2.52	0.00	2.23	0.73	0.00	-3.40	1.00	0.00	0.03
S3026_12283	0.98	1.70	0.02	2.08	0.71	1.00	2.47	0.00	2.22	0.72	0.00	-3.70	1.00	0.00	0.03
S303_48932	0.98	1.62	0.02	2.06	0.71	1.00	2.74	0.00	2.21	0.72	0.00	-3.40	1.00	0.00	0.03

Appendix 5-3: (continued)

	C. fumiferana vs. C. biennis					*C. fumiferana vs. C. occidentalis*					*C. occidentalis vs. C. biennis*				
Locus	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst	Prob	log10(PO)	qvalue	α	Fst
S3167_5715	0.97	1.56	0.02	2.06	0.70	1.00	2.52	0.00	2.23	0.73	0.00	-3.10	0.99	0.00	0.03
S322_48000	0.98	1.60	0.02	2.05	0.70	1.00	2.40	0.00	2.18	0.72	0.00	-3.40	1.00	0.00	0.03
S3548_16668	0.00	-3.70	0.97	0.00	0.27	0.00	-2.70	0.51	0.00	0.27	0.96	1.38	0.02	2.01	0.19
S357_34790	0.16	-0.71	0.20	0.30	0.34	1.00	2.58	0.00	2.23	0.72	0.00	-3.70	1.00	0.00	0.03
S3903_12579	0.97	1.58	0.02	2.06	0.70	0.99	2.28	0.00	2.21	0.72	0.00	-3.00	0.98	0.00	0.03
S4238_9728	0.06	-1.20	0.24	0.11	0.30	1.00	2.55	0.00	2.23	0.72	0.00	-3.70	1.00	0.00	0.03
S490_34259	0.97	1.49	0.03	2.05	0.70	1.00	2.85	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S491_35145	0.97	1.45	0.03	2.05	0.70	1.00	2.34	0.00	2.19	0.72	0.00	-3.40	1.00	0.00	0.03
S495_60381	0.47	-0.05	0.07	0.86	0.46	0.92	1.07	0.01	1.79	0.64	0.00	-3.22	0.99	0.00	0.03
S5435_7373	0.98	1.65	0.02	2.08	0.71	1.00	2.37	0.00	2.21	0.72	0.00	-3.10	0.99	0.00	0.03
S554_58465	0.97	1.48	0.03	2.04	0.70	1.00	2.58	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S5626_8666	0.97	1.59	0.02	2.06	0.70	1.00	2.70	0.00	2.23	0.72	0.00	-3.22	0.99	0.00	0.03
S573_32737	0.01	-1.95	0.31	0.02	0.28	1.00	2.66	0.00	2.21	0.72	0.00	-1000.00	1.00	0.00	0.03
S597_13132	0.98	1.69	0.02	2.06	0.71	1.00	2.40	0.00	2.21	0.72	0.00	-3.00	0.98	0.00	0.03
S6247_11354	0.98	1.61	0.02	2.06	0.70	1.00	2.74	0.00	2.22	0.72	0.00	-3.22	0.99	0.00	0.03
S6461_2674	0.97	1.51	0.02	2.05	0.70	1.00	2.55	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S7737_10848	0.97	1.51	0.02	2.05	0.70	1.00	2.42	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03
S7760_2100	0.97	1.56	0.02	2.07	0.71	1.00	2.58	0.00	2.22	0.72	0.00	-3.22	0.99	0.00	0.03
S8077_3907	0.98	1.61	0.02	2.08	0.71	1.00	2.66	0.00	2.23	0.73	0.00	-3.22	0.99	0.00	0.03
S828_40355	0.97	1.56	0.02	2.05	0.70	1.00	2.66	0.00	2.21	0.72	0.00	-3.40	1.00	0.00	0.03
S851_8607	0.97	1.52	0.02	2.05	0.70	1.00	2.62	0.00	2.22	0.72	0.00	-3.40	1.00	0.00	0.03
S8521_1582	0.97	1.56	0.02	2.05	0.70	1.00	2.80	0.00	2.24	0.73	0.00	-1000.00	1.00	0.00	0.03
S8537_2842	0.98	1.61	0.02	2.07	0.71	1.00	2.74	0.00	2.21	0.72	0.00	-3.70	1.00	0.00	0.03
S869_20836	0.97	1.45	0.03	2.03	0.70	1.00	2.40	0.00	2.19	0.72	0.00	-3.00	0.98	0.00	0.03
S942_9301	0.54	0.07	0.05	1.00	0.49	1.00	2.70	0.00	2.21	0.72	0.00	-3.22	0.99	0.00	0.03

Appendix 5-4: Geographic cline statistics for cline between *Choristoneura fumiferana* and *C. occidentalis* reference populations (Brightsands Lake Regional Park, Saskatchewan and Merritt, British Columbia, respectively). *c* is cline centre (in km from Merritt), *w* is cline width (km), *pL* and *pR* are the allele frequency estimates at the ends of the cline where *pR* is *C. fumiferana*. Parentheses denote two-unti support limits around each estimate.

Locus	c	w	pL	pR
S1_781981	467.7 (453.7-478)	80 (61-95.4)	0 (0-0)	1 (0.9-1)
S1028_38265	461.5 (448.7-473.7)	76.4 (60.7-99.5)	0 (0-0)	1 (0.9-1)
S1079_27608	464.1 (450.7-479.9)	64.8 (50.3-76)	0 (0-0)	0.9 (0.8-1)
S1083_32522	468.6 (457.1-478.7)	96.6 (76.7-116.4)	0 (0-0)	1 (0.9-1)
S1093_15778	466.6 (456.1-477.7)	97.7 (75.9-123.2)	0 (0-0)	1 (0.9-1)
S1103_19660	476.9 (458.6-491.3)	104.6 (74.4-137.6)	0 (0-0)	1 (0.9-1)
S11083_2551	466.8 (454.6-478.2)	91.8 (73.2-115.8)	0 (0-0)	1 (0.9-1)
S11686_2436	466.4 (455.4-476.5)	90.2 (72.5-112)	0 (0-0)	1 (0.9-1)
S11692_839	471.8 (459.1-484.2)	89.9 (71.3-112.9)	0 (0-0)	1 (0.9-1)
S118_99416	468.2 (457.3-479.4)	95.3 (77.4-117.2)	0 (0-0)	1 (0.9-1)
S1255_32680	461.7 (448.3-474.8)	86.8 (67-111.8)	0 (0.2-0)	0.9 (0.8-1)
S12567_3585	466.9 (455.4-479.4)	92.6 (71.4-115.9)	0 (0-0)	1 (0.9-1)
S128_32317	467.9 (457.3-480)	96.4 (75.4-121.7)	0 (0-0)	1 (0.9-1)
S1303_37993	464.5 (449.4-480.1)	93.4 (64.1-129.9)	0 (0-0.1)	0.9 (0.8-1)
S1329_15460	469.3 (453.6-482.7)	98.3 (71.5-125.3)	0 (0-0)	1 (0.9-1)
S1350_14523	467.8 (453.8-481.8)	89.4 (64.3-120.2)	0 (0-0)	0.9 (0.9-1)
S1358_32156	463.4 (449.1-477.4)	80.4 (59.4-105.8)	0 (0-0)	0.9 (0.8-1)
S138_52482	466.1 (454.4-475.9)	91.1 (71.4-100.2)	0 (0-0)	1 (0.9-1)
S1405_2955	471.5 (460.3-481.7)	97.8 (78.3-118.2)	0 (0-0)	1 (0.9-1)
S1429_30415	466.6 (448.8-482)	90.8 (64.7-118.4)	0 (0-0)	0.9 (0.8-1)
S153_73591	468.5 (457.9-478.6)	94.3 (77.6-113.4)	0 (0-0)	1 (0.9-1)
S1555_20885	466.9 (455.7-477.4)	92.2 (73.5-113.6)	0 (0-0)	1 (0.9-1)
S162_91561	473 (451.5-491.8)	108.3 (71.3-143.3)	0 (0-0)	0.9 (0.8-1)
S1623_221	462.8 (449.7-477.4)	72.4 (59.3-98.5)	0 (0-0)	0.9 (0.8-1)
S16237_2280	489.3 (468.9-505.4)	137.1 (93.9-186.2)	0 (0-0.1)	1 (0.9-1)
S16441_2781	471.8 (456.1-485.5)	111.8 (82.4-138.6)	0 (0-0)	1 (0.9-1)
S167_76090	473.1 (459.9-481.3)	67.1 (55.1-76.8)	0 (0-0)	1 (0.9-1)
S1701_6968	470.9 (451.4-489.8)	106.7 (72.4-141.6)	0 (0-0)	0.9 (0.7-1)
S17144_643	466.1 (455.1-476.2)	91.6 (72.5-111.9)	0 (0-0)	1 (0.9-1)
S176_79847	433.1 (427-466.3)	34 (26.5-82.3)	0 (0-0)	0.8 (0.7-0.9)
S1799_22447	449.1 (430-465.2)	44.5 (25.2-62.9)	0 (0-0)	0.9 (0.8-1)

Locus	c	w	pL	pR
S18_76919	466.8 (453.5-479.9)	72.2 (56.2-92.5)	0 (0-0)	1 (0.9-1)
S1816_1019	460.4 (449.7-481.4)	63.5 (53.3-100.2)	0 (0-0)	0.9 (0.8-1)
S184_80842	463.5 (450.4-475.7)	91.1 (68.7-119)	0 (0-0)	1 (0.9-1)
S1855_18882	475.6 (459.3-490.4)	113.1 (79.7-151.7)	0 (0-0.1)	1 (0.9-1)
S19_74531	469.5 (456-479.4)	96 (74.6-116.4)	0 (0-0)	1 (0.9-1)
S19210_2344	460.9 (449.1-474.1)	62 (51.6-81.8)	0 (0-0)	1 (0.9-1)
S1947_11336	461.4 (448.2-474.5)	87.1 (64.2-112.9)	0 (0-0)	0.9 (0.8-1)
S201_49613	460.7 (448.7-472.7)	107.9 (87.2-127.5)	0 (0-0)	0.9 (0.9-1)
S202_52326	472.2 (454.9-486.3)	80 (59-104.3)	0 (0-0)	0.9 (0.8-1)
S203_48267	460.7 (446.4-470.5)	62.3 (47-70.6)	0 (0-0)	0.9 (0.8-1)
S2079_29725	467 (455.4-477.7)	98.3 (77.8-122.6)	0 (0-0)	1 (0.9-1)
S21613_1552	477 (458-493)	103.9 (68.7-139.7)	0 (0-0)	0.9 (0.8-1)
S218_61547	472.8 (460.6-483.2)	111 (89.8-138.4)	0 (0-0)	1 (0.9-1)
S223_67959	466.4 (454.7-476.9)	90.6 (71.8-109.9)	0 (0-0)	1 (0.9-1)
S224_58616	470.9 (459.7-481.4)	97.6 (79.6-118.4)	0 (0-0)	1 (0.9-1)
S2244_19210	467 (453.3-476.6)	90.3 (70.6-105.1)	0 (0-0)	1 (0.9-1)
S22460_2043	466.4 (455.4-476.3)	94.2 (76.2-114.2)	0 (0-0)	1 (0.9-1)
S2252_13326	465.8 (454.7-476)	92 (73.6-110.5)	0 (0-0)	1 (0.9-1)
S2256_13845	468.9 (457.5-481.3)	96.6 (71.7-130.4)	0 (0-0.1)	1 (0.9-1)
S2275_27109	464.5 (453.1-473.9)	90.9 (71.9-110.6)	0 (0-0)	1 (0.9-1)
S22994_1598	473.8 (461.6-486)	81.8 (52-116)	0.1 (0-0.1)	1 (0.9-1)
S2326_19379	465.9 (456-476.1)	92.3 (74.1-111)	0 (0-0)	1 (0.9-1)
S2369_6112	456.9 (444.2-468.2)	60.8 (50.8-70.2)	0 (0-0)	0.9 (0.8-1)
S23725_1679	470.4 (459.4-480.1)	97.5 (79.1-118.3)	0 (0-0)	1 (0.9-1)
S2392_9378	462.8 (439.6-482.9)	82.1 (49.4-114.9)	0 (0-0)	0.9 (0.7-1)
S23936_1965	469 (457.3-481.7)	92.7 (67.6-119.6)	0 (0-0.1)	1 (0.9-1)
S24056_940	466 (449.3-480.4)	77.7 (50.5-106.4)	0 (0-0)	0.9 (0.8-1)
S242_75929	467.4 (454.5-478.1)	92.6 (72.2-113.6)	0 (0-0)	1 (0.9-1)
S2429_7013	466.9 (455.1-477.9)	93.9 (72.7-117.2)	0 (0-0)	1 (0.9-1)
S2440_5725	464 (452.4-475.1)	94 (70.3-120.4)	0 (0-0.1)	1 (0.9-1)
S24945_1546	472.1 (457.6-482.9)	112.7 (88.7-133.7)	0 (0-0)	1 (0.9-1)

Appendix 5-4: (continued)

Locus	c	w	pL	pR
S25_142156	467.6 (452.2-481.3)	96.2 (72.4-122.9)	0 (0-0)	0.9 (0.8-1)
S25324_1726	470.8 (457.1-483.6)	91.7 (69.4-117.1)	0 (0-0)	1 (0.9-1)
S2570_21156	465.3 (453.6-475.6)	91.4 (71-112.6)	0 (0-0)	1 (0.9-1)
S26_66001	478.1 (464.5-488.6)	109.8 (87.5-133.6)	0 (0-0)	1 (0.9-1)
S2634_4417	466.8 (456.3-478.2)	99.6 (75.3-126.3)	0 (0-0)	1 (0.9-1)
S264_75686	466.5 (452.3-476.5)	80.7 (57.4-98.6)	0 (0-0)	1 (0.9-1)
S2796_23807	471.2 (457.8-481.7)	87.4 (67.3-106.7)	0 (0-0)	1 (0.9-1)
S281_34109	465.7 (455.6-476.6)	110.9 (100.4-135.3)	0 (0-0)	1 (0.9-1)
S2956_7939	468.6 (456.1-481.6)	100.1 (77.3-124.3)	0 (0-0)	1 (0.9-1)
S29790_63	433.3 (427.3-438.9)	31.5 (23.3-34.4)	0 (0-0)	0.8 (0.8-0.9)
S300_26415	466.7 (455.4-477.5)	91.2 (72.9-112.7)	0 (0-0)	1 (0.9-1)
S3026_12283	466.1 (454.9-477.7)	105.1 (76.7-137.7)	0 (0-0)	1 (0.9-1)
S303_48932	468 (456.5-479.4)	96.4 (78.2-118)	0 (0-0)	1 (0.9-1)
S3167_5715	465.8 (453.3-476.9)	85.7 (66-105.2)	0 (0-0)	1 (0.9-1)
S322_48000	465.8 (454.7-476)	92 (73.6-110.5)	0 (0-0)	1 (0.9-1)
S351_41429	439.5 (429.5-450.3)	40.5 (28.9-45)	0 (0.1-0)	0.9 (0.8-0.9)
S3513_1258	458.9 (447.3-470.9)	90 (72.6-110.5)	0 (0-0)	0.9 (0.8-1)
S357_34790	466.5 (454.9-477)	91.9 (69.7-115.8)	0 (0-0.1)	1 (0.9-1)
S3903_12579	465.5 (454.1-475.1)	92.5 (73.3-112.6)	0 (0-0)	1 (0.9-1)
S410_60368	448.4 (435.5-465.8)	38.5 (30.2-49.4)	0 (0-0)	0.8 (0.7-0.9)
S4238_9728	467.7 (452.1-481.8)	92.9 (68.1-118.1)	0 (0-0)	0.9 (0.8-1)
S4407_15208	466.5 (455.6-476.9)	97.5 (75.7-121.7)	0 (0-0)	1 (0.9-1)
S4480_15771	465.7 (452.8-477.8)	70 (59.5-77.8)	0 (0-0)	0.9 (0.8-1)
S460_28007	469.4 (454.8-484)	88.1 (58.9-123.6)	0 (0-0)	0.9 (0.8-1)
S490_34259	468.7 (456.7-478.9)	88.6 (69.7-110.3)	0 (0-0)	1 (0.9-1)
S491_35145	461 (448.7-472.4)	85.9 (66.8-102.3)	0 (0-0)	0.9 (0.8-1)
S495_60381	473 (460.8-485.2)	108.9 (86.3-136.2)	0 (0-0)	1 (0.9-1)
S5169_13231	446.9 (431.7-463.8)	49.6 (29.9-67.7)	0 (0-0)	0.8 (0.7-0.9)
S54_54726	467.6 (454.5-479.8)	91 (69.5-115.7)	0 (0-0)	1 (0.9-1)

Locus	c	w	pL	pR
S5435_7373	465.4 (453.9-476)	94.5 (74.7-115.4)	0 (0-0)	1 (0.9-1)
S554_58465	467 (455.3-478)	94.6 (74.6-115.2)	0 (0-0)	1 (0.9-1)
S5626_8666	466.9 (455.7-477.4)	96.2 (76.7-116.2)	0 (0-0)	1 (0.9-1)
S573_32737	464.7 (451.8-476.3)	109 (84.4-136)	0 (0-0.1)	1 (0.9-1)
S597_13132	473.8 (461.8-483.7)	98.2 (83.5-120.3)	0 (0-0)	1 (0.9-1)
S6230_5330	470 (454.5-482.8)	101.5 (72.6-135.3)	0 (0-0.1)	1 (0.9-1)
S6247_11354	467.6 (456.2-477.7)	90.7 (72.9-111.3)	0 (0-0)	1 (0.9-1)
S6461_2674	470.5 (459.8-481)	100.1 (81.3-121.7)	0 (0-0)	1 (0.9-1)
S6568_8142	460.7 (445.4-474.5)	96.3 (65.4-124.8)	0 (0-0)	1 (0.9-1)
S67_97681	467.6 (450-484.8)	81.7 (55.2-111.7)	0 (0-0)	0.9 (0.8-1)
S726_37547	460.4 (445.6-471.7)	86.9 (63-103.5)	0 (0-0)	0.9 (0.8-1)
S7737_10848	465.4 (452.3-475.4)	86.2 (66.1-105.5)	0 (0-0)	1 (0.9-1)
S7760_2100	467.2 (455.8-476)	91.1 (73.7-110.5)	0 (0-0)	1 (0.9-1)
S7825_2328	471.4 (459.3-484.1)	94.1 (67.4-122.2)	0.1 (0-0.1)	1 (0.9-1)
S801_17522	470.3 (457-483.9)	100.7 (73.7-134.8)	0 (0-0)	1 (0.9-1)
S8077_3907	468.4 (456.9-480.3)	106 (77.6-137.2)	0 (0-0)	1 (0.9-1)
S8193_8770	467.4 (455.4-480.3)	98.7 (69.7-132.9)	0.1 (0-0.1)	1 (0.9-1)
S828_40355	467.9 (455.5-478.4)	85.2 (67.8-104.9)	0 (0-0)	1 (0.9-1)
S8367_1591	477.7 (465.1-489.4)	103.7 (82-129.6)	0 (0-0)	1 (0.9-1)
S851_8607	469.2 (457.2-479.1)	99.2 (77.8-121.2)	0 (0-0)	1 (0.9-1)
S8521_1582	469.6 (455.6-479.7)	78.1 (56.6-94.4)	0 (0-0)	1 (0.9-1)
S8537_2842	465.2 (453.7-475.2)	93.5 (74.9-112.8)	0 (0-0)	1 (0.9-1)
S869_20836	455.4 (440.6-469.3)	69.3 (50.3-95.2)	0 (0-0)	0.9 (0.8-1)
S8765_174	461.8 (441.8-473.7)	62.9 (41.7-78.7)	0 (0-0)	0.9 (0.8-1)
S9_78967	466.2 (454.1-478.4)	109.9 (84.8-135.2)	0 (0-0)	1 (0.9-1)
S931_1800	468 (454.8-480.4)	96.6 (71.1-127.1)	0 (0-0)	1 (0.9-1)
S9330_5448	461.9 (447.9-475)	84.6 (64.6-112.4)	0 (0-0)	1 (0.9-1)
S942_9301	468.9 (457.3-479.7)	99.1 (77.2-122.8)	0 (0-0)	1 (0.9-1)
S98_26822	469.3 (455.5-483.3)	101.7 (74.5-135)	0 (0-0)	1 (0.9-1)

Chapter 6

Preliminary genetic linkage map for the spruce budworm species complex (*Choristoneura fumiferana,* Lepidoptera: Tortricidae)

Introduction

The availability of dense genetic linkage maps allows one to address some of the more elusive questions in evolutionary research, such as identifying the genomic architecture of species differences. To that end, genetic maps have been used extensively as a foundation for mapping species differences that follow simple Mendelian inheritance, such as insecticide resistance (Heckel *et al* 1999; Baxter *et al*. 2011) and wing colour variation (Beldade *et al*. 2009; Kapan *et al*. 2007) in Lepidoptera, as well as complex quantitative trait loci in other organisms, such those underlying variation in wing size (Gadau *et al*. 2002) and sex-pheromone composition (Groot *et al*. 2013), or reproductive morphology, life history and physiology (Bradshaw *et al.* 1998; Lexer *et al*. 2005). More recently, genetic linkage information has been an integral component of genome scans used to identify tracts of genome sequence that are subject to selection (Turner *et* al. 2005; Via and West 2008; Nosil *et al*. 2009; Via 2012; Strasburg *et al*. 2012).

However, the ability to obtain high quality linkage maps can be hampered by difficulties in developing more traditional types of genetic markers, such as microsatellites which typically require extensive laboratory work to develop for organisms without available genomic resources (Lumley *et al*. 2011; Beldade *et al*. 2009). In addition, some biological systems are more prone to complications based on the characteristics of their genomes. For instance, Lepidoptera have a large number of chromosomes that can make linkage mapping difficult (Ennis 1976; Wang and

Porter 2004), and are also known to contain large amounts of transposable elements that can complicate marker development (Zhang 2004; van't Hof *et al*. 2007; Sinama *et al*. 2011).

The recent development of high-throughput genotyping methods, such as Restriction-enzyme Associated DNA sequencing (RADseq) and Genotyping-By-Sequencing (GBS), that take advantage of next-generation sequencing platforms, has opened the doors to genomics for non-model organisms (Miller et al 2007; Baird et al 2008; Elshire et al. 2011; Davey et al 2011). In particular, these methods have made development of linkage maps for non-model organisms a possibility. Indeed, much of the research describing these techniques has focused on that particular goal (Baird *et al*. 2008; Andolfatto *et al*. 2011; Elshire *et al*. 2011; Poland *et al*. 2012). These approaches employ a suite of individual-specific sequence barcodes to enable pooling of restriction enzyme-digested genomes of multiple individuals into a single sequencing lane. This allows millions of orthologous sequences to be read across the pool of individuals. Through subsequent *de novo* assembly of the sequenced reads or alignment to a known reference genome (Elshire *et al*. 2011; Catchen *et al*. 2011) the resulting sequence data can be used for variant discovery and genotyping. One particularly useful aspect of reference guided marker discovery is that it can be coupled with development of linkage maps to order loci embedded in reference scaffolds along chromosomes to improve genome assembly. For instance, such approaches have been employed to verify proper assembly in other lepidopterans (Rastas *et al*. 2013; Yammamoto *et al*. 2008), including the lepidopteran reference, *Bombyx mori*.

The spruce budworm, *Choristoneura fumiferana*, species complex consists of some of North America's most economically important forest pests (Volney and Fleming 2007). Recently, interest in characterizing the genomic composition of spruce budworms has been spurred by a need to find alternatives to the subtle differences in the life history and morphology

that have been previously used for identification of these species (Harvey 1985; Powell and De Benedictis 1995). An early draft genome assembly has been developed for *C. fumiferana* but it remains incomplete due to difficulties in establishing linkage relationships among its 30,517 scaffolds (Spruce Budworm Consortium, assembled 19 April 2011). Here, we use GBS to (i) identify thousands of variable loci throughout the spruce budworm genome, (ii) develop a preliminary genetic linkage map for spruce budworms in an attempt to help establish linkage relationships among reference scaffolds for validation of future assemblies of the genome, (iii) examine the extent of chromosomal synteny between the spruce budworm and the silkworm *Bombyx mori*, and (iv) identify putative protein coding loci among those mapped in the spruce budworm. We use the existing genome assembly as a reference sequence for GBS read alignment and marker discovery in a hybrid mapping family of two closely-related spruce budworms, *Choristoneura biennis* (Freeman) and *Choristoneura occidentalis* (Freeman). Although the latter was recently renamed *Choristoneura freemani* Razowski (Razowski 2008), wide-spread adoption of this new name has not been observed (Gilligan and Brown 2014). Consequently, we maintain consistency with the extensive literature for the older name, *C. occidentalis*.

Methods

Crosses

We used a mapping family consisting of an outbred interspecific hybrid backcross between *C. occidentalis* and *C. biennis* that originated from diapause work conducted by Nealis (2005). Both parents of the backcross and 48 of their progeny (28 males and 20 females) were

used. The recombinant F1 parent was a male (*i.e.* male-informative backcross, BC234) and was backcrossed to an unrelated outbred female of its father's species (*i.e. C. occidentalis*). Thus, the number of useful loci is expected to have been eroded by a reduced proportion of shared alleles between the parental generation and the backcrossed parental individual. This, however, should result in a backcross generation in which alleles at different loci segregate relative to either parent, but with greater numbers of informative loci deriving from the F1 male. In addition, since all samples originated from outbred populations, high levels of heterozygosity among the parental generation may reduce the number of loci with detectable segregation patterns among the backcrossed progeny and may potentially lead to difficulties identifying linkage relationships across whole chromosomes.

GBS Library Preparation and Sequencing

Leg and thoracic tissue from the parents and progeny were used for isolating RNA-free DNA using a DNeasy Blood & Tissue DNA purification kits (Qiagen). DNA was suspended in Millipore water and shipped to the Institut de biologie intégrative et des systèmes (IBIS) at the Université Laval (Quebec City, QC) where 96-plexed GBS libraries were prepared. Library preparation followed the protocol of Poland *et al*. (2012) using the *PstI-MspI* enzyme combination, but was modified slightly as follows: 600ng of the pooled, adapter-ligated and amplified library was treated with duplex-specific nuclease to remove the repetitive fraction of the library (Zhulidov *et al.* 2004); and an additional PCR step using a selective reverse primer extending a single base (C) past the 3' restriction site into the library inserts was used to selectively amplify one-quarter of the total number of fragments (Sonah *et al*. 2013). The library

was then single-end sequenced to 100 bases on an Illumina HiSeq2000 sequencer at the McGill University-Génome Québec Innovation Centre in Montreal, QC.

Data Processing

Raw GBS reads were split into individual-specific datasets using the Fastx Barcode Splitter (v. 0.0.13, http://hannonlab.cshl.edu/fastx_toolkit/). Only exact matches to barcode adaptors were retained (see Table 6-1 for a list barcode adapters). Barcode adapters were then trimmed from each sequence using the Fastq Trimmer (v. 0.0.13, http://hannonlab.cshl.edu/fastx_toolkit/). The Burrows-Wheeler Aligner (BWA v. 0.6.2-r126, Li and Durbin 2009) was used to align barcode-trimmed reads from each individual to the draft spruce budworm genome. Bases with quality scores below 10 were soft-trimmed during the alignment process. Samtools (v. 0.1.19-44428cd, Li *et al.* 2009) was then used to generate variant locus pileups for each mapping family (parents + progeny) using only reads with a mapping quality greater than 10. To correct for excessive base mismatches resulting from misalignment around indels, the coefficient for downgrading mapping quality was used (-C50). Variants were then mined using custom awk scripts (provided by P. Rastas, Nov. 18, 2013) to retain only SNP loci that had a minimum total read count equal to 3 times the size of the family with a maximum of 15% of individuals with coverage less than 3 reads, and a minor allele frequency greater than 0.2, as in Rastas *et al*. (2013). Allele counts for each of the resulting SNP loci were then tabulated and the data was formatted for input into the genetic mapping software Lep-Map (v. 0.2, Rastas *et al.* 2013).

Identifying Chromosomes

Since most Lepidoptera exhibit achiasmatic oogenesis and female heterogamety (WZ:ZZ), whereby recombination only occurs in males (Ennis 1976; Suomalainen *et al*. 1973), a 2-phase approach was employed to conduct linkage mapping (Rastas *et al.* 2013; Baxter *et al*. 2011; Beldade *et al.* 2009; Heckel *et al.* 1999). This approach takes advantage of the complete linkage expected between maternally-informative (*i.e.* heterozygous in the mother) loci and allows for linkage groups to be determined by looking for maternally-informative loci with identical segregation patterns. Following linkage group assembly, male-informative loci anchored on these maternal linkage groups can then be used to order markers. Both linkage grouping and marker ordering were carried out using Lep-Map v. 0.2 (Rastas *et al.* 2013). This software is specifically designed to handle linkage mapping in lepidopteran systems and implements a number of features useful for large datasets where missing data may present challenges (eg. imputation of genotypes).

Locus allele counts were converted into genotypes using Lep-Map's Counts2Genotypes module using default settings and allowing read error estimates to be automatically determined. Maternally-informative loci (*i.e.* heterozygous in the mother) were assigned to linkage groups with a pairwise LOD score of 6.2 ($p = 0.04$, as determined by the EstimateLODLimit module), joining loci with similar/complementary segregation patterns across the progeny. Any locus with significant ($p < 0.05$) deviation from expected Mendelian allele proportions was removed from the dataset during this process. Here, we define segregation patterns as strings of 0s and 1s representing the alternate parental alleles present in each progeny. Since the spruce budworm has 30 chromosomes (2n=60, Ennis 1976), we would expect to find the same number of complementary segregation patterns (Rastas *et al.* 2013; Baxter *et al.* 2011). However, the

identification of correct and erroneous linkage groups presented challenges due to the low and similar numbers of assigned markers across all groupings. Subsequently, in an effort to maximize chromosome coverage we retained all linkage groups with a minimum of six loci per group rather than keeping only the 30 largest linkage groups. Finally, any ungrouped maternally-informative markers remaining with multi-point LOD scores greater than 5.3 ($p = 0.04$, determined with EstimateLODLimit with maxLodLimit=6.2) to loci in any of the 53 linkage groups were added using the JoinSingles module.

Chromosomal Maps

To increase marker density on individual linkage groups and facilitate the ordering of loci within linkage groups, paternally-informative loci (heterozygous in the father) were also grouped into identical/complementary segregation patterns using a pairwise LOD score of 5.6 ($p = 0.05$) and a size limit of at least four loci. JoinSingles was again used to augment marker densities by assigning additional paternally-informative loci to paternal linkage groups with a multi-point LOD score of 4.6 ($p = 0.03$). ScaffoldHMM was then used to probabilistically assign all linkage groups (maternal and paternal) to the maternal linkage map, and only assignments with a LOD score greater than one were retained. Where possible, ordering of paternally-informative loci on linkage groups was performed using the OrderMarkers module first without error modelling (*i.e.* learnErrorParameters=0). Loci with genetic distances greater than 50cM from the next ordered locus were then discarded as potential errors in marker ordering. This was especially evident where loci from the same reference scaffold (largest scaffold = 822527 bases) were positioned in disparate blocks of ordered markers instead of near to each other. In such cases, the ordered

block that included the largest number of paternal loci within a linkage group and largest genetic distance was treated as the correct order. OrderMarkers was then rerun using the remaining paternally-informative loci with error modelling to complete marker ordering.

Chromosome homology and synteny with Bombyx mori

To narrow the number of maternal linkage groups to the expected 30 chromosomes for spruce budworms, we exploited the known high degree of synteny among lepidopteran genomes (Baxter *et al.* 2011; Beldade *et al.* 2009; Pringle *et al.* 2007; Yasukochi *et al.* 2006) by employing homology searches to identify the "putative" chromosomal origins of loci on each linkage group. The VCFtools (v. 0.1.12a, Danecek *et al*. 2011) perl module *fill-fs* and custom bash shell scripts were used to extract up to 1000 bases of flanking sequence from the spruce budworm reference genome for each locus assigned to maternal linkage groups. Flanking sequences were then queried against the assembled genome of *Bombyx mori* (integrated sequence, published 30 September 2008, accessed 6 June 2014 from http://sgp.dna.affrc.go.jp) using tblastx (1e-10), as well as, the UniRef90 protein database using blastx (1e-10). Homology search results on the *B. mori* genome were filtered using the methods of Baxter *et al.* (2011) so that only loci with unambiguous hits to *B. mori* chromosomes were used in making chromosome assignment inferences. Maternal linkage groups were then given the chromosome designation that was most represented among independent (*i.e.* from different spruce budworm scaffolds) loci with unambiguous blast hits or, in cases where a tie was observed, the designation which yielded the most significant e-value. To deduce the degree of synteny of the spruce budworm linkage map with that of *B. mori*, each ordered marker was paired with the genetically mapped *B.*

mori locus (accessed 21 June 2014 from http://sgp.dna.affrc.go.jp) (see Appendix 6-1) residing nearest to the start of the matching sequence in the best blast hit *B. mori* chromosome region. The genetic positions of the paired loci in the spruce budworm were then compared to the *B. mori* genetic maps.

Results

GBS Data Filtering

GBS sequencing of the single backcrossed mapping family resulted in a total of 291,501,100 raw 100 base reads. Of these, 13,659,947 were successfully trimmed of barcode sequence and mapped to the spruce budworm reference genome for an average of 278,193.9 reads per individual (Table 6-1). A single individual (BC234-48) had low sequencing coverage (number of mapped reads below two standard deviations of the mean) and was removed for the remaining analyses. Among mapped reads, a total of 9710 loci were discovered that passed our variant locus filtering pipeline. Of these, 883 were indels and were discarded from the dataset resulting in a total of 8827 SNPs for linkage mapping.

Linkage Mapping

Allele count data was successfully converted into genotypes for 7342 SNP loci using a genotyping error rate of 0.025 as estimated automatically from the data by Lep-Map. This posits that the remaining markers could not be genotyped in the parents and therefore were of no use to linkage analysis. This occurs if the log-odds difference in likelihood between the best and

second-best parent genotype combinations is less than two. The number of informative loci deriving from the mother and father was 1830 and 2098, respectively, with 1543 markers heterozygous in both parents. Clustering of maternally- informative loci into groups with identical or complementary segregation patterns resulted in 53 linkage groups with between 6-50 markers for a total of 967 (Table 6-2). The sex (Z) chromosome was identified as maternal linkage group 52 based on the observation of all female progeny being homozygous for paternally-derived alleles at 6 loci. Performing the same analysis using only paternally-informative markers resulted in 77 linkage groups with overall greater numbers of loci (4-55 per group and 1376 total) as expected from the mating configuration of the backcross. The merger of these separate maternal and paternal maps using the maternal map as a backbone identified 2638 unique spruce budworm scaffolds among the two maps (Appendix 6-1). However, only 578 (361 maternal and 392 paternal) scaffolds and 1290 markers (575 maternal and 715 paternal) could be assigned to maternal linkage groups with a LOD>1. Since marker ordering within linkage groups relies solely on paternal markers due to recombination being present in males only within spruce budworms, a much smaller fraction of the paternally-informative markers could actually be ordered (*i.e.* 438) and that had genetic distances less than 50cM from other markers within linkage groups (Table 6-2). Consequently, markers on only 37 linkage groups could be ordered, each with a map length between 3.13-82.43cM (for total map length of 990.44cM) and between 3-44 markers (Table 6-2).

Chromosome Homology

The inability to narrow linkage group numbers down to 30 based on marker density and segregation patterns alone required assistance by other means to help identify their chromosomal identities, with the exception of the Z chromosome. We were able to obtain homology-based chromosomal designations for 50 of 53 maternal linkage groups matching to 26 *B. mori* chromosomes (2-15 and 17-28) (Table 6-3). For each of these at least a single blast hit with significant e-value was obtained through our tblastx searches with only 24 linkage groups matching uniquely to a single *B. mori* chromosome (Table 6-3). From these, 220 paternally-informative loci were ordered along maternal linkage groups and were available for examination of chromosomal synteny with *B. mori*. Figure 6-1 illustrates the extent of synteny between these two species. Through this examination we found several instances where spruce budworm linkage groups may contain errors in the form of potentially non-chromosomal locus pairs occurring on the same linkage group, for instance when two loci mapped to the same spruce budworm linkage group are homologous with different *B. mori* chromosomes. This was evident for 17 of the 26 putative spruce budworm chromosomes, and for several of them this apparent non-synteny was extensive (chromosomes 11, 14, 17, 23 and the sex chromosome 52, see Figure 6-1). Despite the potential for error in chromosome assignment it is especially noteworthy that most other chromosome designations appear to contain linkage groups with conserved marker order with homologous regions in the *B. mori* genome (*i.e.* chromosomes 6-8, 10, 12, 13, 15, 18-21, 24, 27-28). Regardless, the ordering of markers in most maternal linkage groups and *B. mori* chromosomes is comparable, suggesting a high degree of synteny between these species.

Of the 1290 loci on the merged map, we were able to identify 48 loci with significant homology to known protein-coding genes (Table 6-3). All blastx hits to the UniRef90 protein

database yielded best hits to lepidopteran proteins with e-values greater than 4e-11, with the exception of a single match to a protein within the pancrustacea (Appendix 6-2).

Discussion

Although there has been much interest and work on the genetic characteristics of spruce budworm phenotypes since its redescription in the genus *Choristoneura* by Freeman (1947), no attempt has previously been made to develop a genetic linkage map. Through the use of GBS, we identified 8827 SNP loci from 49 individuals of an interspecific hybrid backcross between *C. occidentalis* and *C. biennis*. These loci have allowed us to develop the first, preliminary genetic linkage map for spruce budworms, consisting of 53 linkage groups that will be especially important in validation of the future genome assemblies. At present, the draft genome for *C. fumiferana* is composed of 30,517 scaffolds varying from approximately 1,000-800,000 bases. Using this linkage map, 578 of these genome scaffolds were grouped by similarity in the segregation patterns of maternally-informative loci positioned within them, and 438 have been ordered along linkage groups using adjacent paternally-informative loci.

Work conducted by Ennis (1976) established the precise number of spruce budworm chromosomes to be 30 (2n=60), but most Lepidoptera have from 28 to 31 chromosomes with an inferred ancestral number of 31 (Ennis 1976; Lukhtanov 2000; Sichova *et al*. 2013). Although a one-to-one correspondence between linkage groups and expected chromosome numbers was not observed, we were able to use homology-based searches of reference genome sequence flanking each locus to infer the assignment of all but three linkage groups to chromosomes 2-15, and 17-28 of the *B. mori* genome (2n=28). At least one other study (Wang and Porter 2004) has also

reported linkage group numbers well above those expected for Lepidoptera (51 for a 2n=31 system) and attributed this to difficulties in mapping genomes with large numbers of chromosomes. By relating potentially protein-coding spruce budworm sequences anchored on each linkage group to *B. mori* orthologs it was further established that most of these putative chromosome assignments consist of conserved marker orders, and are therefore in agreement with literature citing macrosynteny among the Lepidoptera (Baxter *et al.* 2011; Beldade *et al.* 2009; Pringle *et al.* 2007; Yasukochi *et al.* 2006). Regions where multiple linkage groups were syntenic with a single *B. mori* chromosome (*e.g.* chromosomes 3, 4, 7, and 25) may actually represent independent spruce budworm chromosomes, as expected if the 30 chromosomes in the spruce budworm are reduced to the 28 within the silkworm. Where dubious assignments occur (especially for chromosomes 1, 5, 11, 14, 17, and 23) these may indicate false positive tblastx hits resulting from the spurious alignment of spruce budworm scaffold sequences against the *B. mori* assembled chromosomes (Beldade 2009). Duplicated gene regions and mapping error are also likely factors leading to similar patterns. The criteria employed for identification of the sex-chromosome, in particular, are susceptible to genotype errors resulting from low sequencing coverage, as all markers homozygous in all progeny will be assigned sex-linkage. In addition, a maternal segregation pattern in which alleles segregated according to sex, as in Baxter *et al.*(2011), and therefore of pseudo-autosomal origin, was not detected suggesting that homologous regions of the Z and W sex chromosomes are not present in the linkage map. Similarly, the occurrence of multiple *B. mori* chromosomes assigned to single spruce budworm linkage groups may indicate fusion of non-homologous chromosomes, as is common among the Lepidoptera (Pringle *et al.* 2007; Beldade *et al.* 2009). Examples include the recent discovery of a joint autosomal/sex chromosome origin for the Z and W chromosomes of the Tortricidae (Sichova *et*

al. 2013), and the purported fusions of six ancestral chromosomes in the genome of *Heliconius melpomene* (Pringle *et al*. 2007). Further problems may stem from chromosomal differences among spruce budworm species used; for example Ennis (1976) confirmed the presence of two large and presumably sex chromosomes in *C. fumiferana,* but only a single one in *C. biennis*.

While a complete linkage map for spruce budworms remains to be developed, the map reported here may provide some statistics for comparison with other lepidopteran linkage maps. The total map length we were able to deduce amounted to 990 cM, which is far below the reported range for other lepidopteran systems, again attesting to its preliminary form. For example, map lengths have been reported for *Heliconius melpomene* (1616 cM, Jiggins *et al*. 2005), *Heliconius erato* (1430-2400 cM, Tobler *et al*. 2005; Kapan *et al*. 2006), *Colias* butterflies (2541.7 cM, Wang and Porter 2004), *B. mori* (~2000 cM, *Bicyclas anynana* (1642.2 cM, Beldade *et al*. 2009), *Plutella xylostella* (1292 cM, Baxter *et al*. 2011), and *Melitaea cinxia* (1466-1704 cM, Rastas *et al*. 2013). Among the markers mapped to unique positions along each of our linkage groups we calculated a marker density of approximately 1 per 8.6 cM (range 1.5-50.6 cM across all ordered linkage groups). Also, it is estimated that the linkage map corresponds with a physical map distance of approximately 521kb per cM, as deduced by division of the total size of the spruce budworm genome (~516.4 ±5.5 Mb, Cusson *pers. comm.* 26 July, 2012) by the total map length.

Despite the moderate success we observed in the identification of correct linkage groups with resolved marker positions, mapping may have been hindered by the configuration of the family that was available in this study. The family used a parental generation originating directly from outbred (wild) populations of each species (Nealis 2005), and the backcross generation may itself have introduced unrelated alleles that segregated among the progeny. Thus, the use of an

F2 mapping population may have been a better choice of mapping family since the introduction of new alleles would not occur. Alternatively, the use of inbred strains of each species at each of these mating steps would have vastly improved our ability to detect segregation patterns of maternally-informative markers. However, the mapping family that used here was available from a prior study at no additional cost. Finally, the use of multiple families would have also increased the ability to detect segregating alleles as genotypes at loci may be homozygous in the parents of one family but heterozygous in others (Beldade *et al*. 2009). This would allow for a greater number of informative loci for determine linkage relationships. Future work to improve this map will also require increased number of progeny to obtain finer scale mapping of marker locations, in addition to subsequent sequencing runs to increase read depth for more accurate genotype deduction. Nevertheless, the use of the GBS method has at least allowed important linkage information to be determined for scaffolds within linkage groups, and will contribute to establishing scaffold ordering and serve to help validate future spruce budworm assemblies. Thus the linkage map presented here provides a foundation for additional genomic resources to be built upon, including successive maps with denser marker sampling.

Table 6-1: Mapping family and sequencing information.

Individual	Generation	Sex	GBS Barcode	Sequences
BC234-12	Progeny	Male	TGCGA	281191
BC234-21	Progeny	Male	ACCGT	269586
BC234-8	Progeny	Male	GTAA	351029
BC234-30	Progeny	Male	GTCAA	302024
BC234-17	Progeny	Male	TTCTG	316584
BC234-26	Progeny	Male	CATCT	277456
BC234-35	Progeny	Female	CCACGT	329008
BC234-44	Progeny	Female	ACCTAA	255807
BC234-49	Progeny	Female	CTATTA	260908
BC234-13	Progeny	Male	CGCTT	215162
BC234-22	Progeny	Male	GCTTA	301981
BC234-9	Progeny	Male	AGCG	249776
BC234-31	Progeny	Male	TAATA	356762
BC234-40	Progeny	Female	CTTCCA	182177
BC234-18	Progeny	Male	AGCCG	218276
BC234-27	Progeny	Male	CCTAG	278124
BC234-36	Progeny	Female	TTCAGA	356838
BC234-45	Progeny	Female	ATATGT	209819
BC234-5	Progeny	Male	AACT	285329
BC234-14	Progeny	Male	TCACG	459176
BC234-23	Progeny	Male	GGTGT	241839
BC234-32	Progeny	Male	TACAT	349532
BC234-41	Progeny	Female	GAGATA	240299
BC234-50	Progeny	Female	GCCAGT	216762
BC234-19	Progeny	Male	GTATT	322510
BC234-28	Progeny	Male	GAGGA	313630
BC234-37	Progeny	Female	TAGGAA	276093
BC234-46	Progeny	Female	ATCGTA	280276
BC234-10	Progeny	Male	GATG	249562
BC234-6	Progeny	Male	GCGT	273726
BC234-15	Progeny	Male	CTAGG	247801
BC234-24	Progeny	Male	AGGAT	193054
BC234-33	Progeny	Female	TCGTT	248166
BC234-42	Progeny	Female	ATGCCT	325635
BC234-51	Progeny	Female	GGAAGA	169075
BC234-29	Progeny	Male	GGAAG	213306
BC234-38	Progeny	Female	GCTCTA	238511
BC234-47	Progeny	Female	CATCGT	299912
BC234-11	Progeny	Male	TCAG	369180
BC234-20	Progeny	Male	CTGTA	280434
BC234-7	Progeny	Male	CGAT	228680
BC234-16	Progeny	Male	ACAAA	283968
BC234-25	Progeny	Male	ATTGA	290283
BC234-34	Progeny	Female	GGTTGT	222820
BC234-43	Progeny	Female	AGTGGA	195251
BC234-52	Progeny	Female	GTACTT	217454
BC234-39	Progeny	Female	CCACAA	268520
BC234-3	Father	Male	ACTA	387387
BC234-4	Mother	Female	CAGA	308699
BC234-48*	Progeny	Female	CGCGGT	150569

*Discarded due to low sequencing coverage.

Table 6-2: Maternal linkage groups with locus density and map lengths.

LG	Segregation Pattern	Grouped	Merged	LOD1	Mat	Pat	Ordered	Blocks	Size (cM)
1	00101010111000111100001111011101100011000111010	50	79	24	17	7	4	1	3.13
2	00o0oo01111010i100111o1100010o11100111100o0001o	41	65	17	12	5	0	0	0
3	11000000000100i110?0010i10110100011ii0i110101001	38	62	8	2	6	0	0	0
4	01100100011011110111100111001110010110000001101	38	50	19	12	7	0	0	0
5	01100000100010010101000100001000111011110011010	38	70	16	6	10	10	1	29.64
6	10010101001111001011011111111011010000011111000	36	52	7	4	3	0	0	0
7	11001100111101100111011111111000001110000011100	33	35	18	12	6	6	1	24.54
8	10110110000011011111001110111010110000010011001	30	47	25	8	17	16	1	16.47
9	11100101110110001010101101100000100110100111011	31	48	26	8	18	13	1	23.65
10	11101010111000111101111110000010010110000011110	30	51	30	9	21	0	0	0
11	10000111111001011000110101001001011100001010110	27	37	11	1	10	10	1	11.5
12	10100100110101010011011100110001011000010101110	25	81	55	11	44	44	2	3.45,29.5
13	00011110111100110110000110011101100000111010011	26	43	22	8	14	13	1	44.49
14	01000000110010110101000101101000111000000110001	24	65	29	5	24	0	0	0
15	00011111111111101010000111001011100101011000000	22	53	45	22	23	8	1	35.29
16	00010110011101010010010000111011101010100000011	22	42	18	2	16	15	1	19.5
17	11000011110100001000100011010001101010010010011	21	41	33	21	12	0	0	0
18	00111o0001o11110110101i100i11110111001o01001	20	41	13	11	2	0	0	0
19	1i0110001oo11110101010001101 1o0101101111011 0i00	20	65	28	23	5	3	1	3.45
20	11111100011011011101101010011111001111000110110	20	38	20	12	8	7	1	46.47
21	11101000111100100011100110001111io100111111 0i10	19	29	27	18	9	7	1	4.56
22	11001001110000i11110111011001110o101100011i0i10	18	52	34	21	13	11	1	6.7
23	o0100i111o001ii1o10011io1010o1100000101o01011o1	19	70	50	9	41	41	2	4.88,44.17
24	01010011100000100100110111111110001101010011110	18	37	32	18	14	9	1	72.62
25	00?00110011101000011i000?01110i00?1101100101110	17	44	39	20	19	18	1	25.64
26	10001101o10111000101011111111101111000o0110010o0	16	62	42	16	26	9	1	16.94
27	11111100110100 1o11100110110001101100 00o10011011	16	38	33	9	24	16	1	4.83
28	00010011011111111000000101101010000011110000 1101	16	66	59	16	43	8	1	23.53
29	01i011110101100000100011100010100001100100111i1	15	36	36	15	21	17	1	7.52
30	001o01011000000101011000110110 00o01100010o00i11	14	45	27	15	12	12	2	16.44,42.36
31	10010101010011010000001i000oo00o00111000100i111	13	22	22	14	8	4	1	51.5
32	01011010111001100001101010000010010010010001110	13	30	30	13	17	7	1	25.36
33	111o01000111 0i11011000i011i01i00101000011i11000	12	48	34	12	22	19	1	19.52
34	11001011110010001111 00o0000010o101010010i00000i1	12	65	25	15	10	4	1	11.96

LG refers to the maternal linkage group number; segregation patterns correspond with the consensus of haplotype completion step in Lep-Map's SeparateChromosomes module and represent the maternally-derived alleles (0 and 1) present in each progeny ordered as listed in Table 6-1; o and i represent weaker haplotype consensus resulting from a low number of markers with this allele for an individual; ? represents no clear haplotype consensus for a given individual; Grouped indicates the number of materally-informative loci initially grouped into each LG; Merged indicates the number of maternally and paternally-informative loci in the merged map and Mat and Pat refer to the number of loci with an assignment LOD score>1 from each of the maternal and paternal maps; Ordered indicates the number of ordered paternally-informative markers and Blocks denotes the number of discontinuous segments along LGs (*ie.* loci separated by distances > 50cM); Size indicates the length in cM of each LG block.

Table 6-2: (continued)

LG	Segregation Pattern	Grouped	Merged	LOD1	Mat	Pat	Ordered	Blocks	Size (cM)
35	00000101010101000000111110101010011000 1o0010100	11	48	12	5	7	7	1	5.27
36	10100111111111100010o10100100010000011101110101	10	37	17	12	5	5	1	15.41
37	01011000110101111001011000100000011000111001110	10	27	19	10	9	5	1	13.99
38	00101110010001000001111110i00101100010001111011	9	41	21	11	10	0	0	0
39	00010111100111000o10100010001001 0i111o0101000oi1	10	39	5	5		0	0	0
40	1010o11101111100010110 1o01100100001010100110101	9	38	18	13	5	4	1	4.55
41	01011111101100111110110000010010o11010011111i00	9	46	21	11	10	10	1	82.43
42	11000111101100101011000000011100011000011001110	9	24	24	9	15	13	1	16.27
43	01110011011100111100000101011101100110110001010	10	41	22	2	20	0	0	0
44	00111000110011010111101000000110111010001110001	8	25	17	14	3	0	0	0
45	00111111010101111101011111000101100110011001011	9	31	20	9	11	9	1	28.9
46	00011000110001010111101 0o000011011101010100000 1	8	23	11	7	4	0	0	0
47	11001000011000111100101101111110011010100100000 0	7	31	23	9	14	12	1	6.83
48	11010111101000011010101001010001010000110111100	7	54	16	10	6	0	0	0
49	110111100?110i011i000010010i0100000001111010111	6	24	24	7	17	11	2	5.89,4.45
50	10000101010111000000111010000010011000100000110	6	27	25	6	19	16	2	9.4,18.97
51	00001110010110 0o001i000101101000 00i1?0000011111	6	30	12	6	6	0	0	0
52	11111111111111111111111111111111111111111111111111	6	17	16	6	10	15	4	50.58,8.35,34.55,14.99
53	01100001110111100011100000110001111111111101000	6	25	13	6	7	0	0	0
Total		966	2337	1290	575	715	438		

LG refers to the maternal linkage group number; segregation patterns correspond with the consensus of haplotype completion step in Lep-Map's SeparateChromosomes module and represent the maternally-derived alleles (0 and 1) present in each progeny ordered as listed in Table 6-1; o and i represent weaker haplotype consensus resulting from a low number of markers with this allele for an individual; ? represent no clear haplotype consensus for a given individual; Grouped indicates the number of materally-informative loci initially grouped into each LG; Merged indicates the number of maternally and paternally-informative loci in the merged map and Mat and Pat refer to the number of loci with an assignment LOD score>1 from each of the maternal and paternal maps; Ordered indicates the number of ordered paternally-informative markers and Blocks denotes the number of discontinuous segments along LGs (*ie.* loci separated by distances > 50cM); Size indicates the length in cM of each LG block.

Table 6-3: Chromosome homology between spruce budworm linkage groups and the *Bombyx mori* genome.

BmChr	LG	Hits	Other Hits	UniRef90
1 (Z)*	52	-	4(1), 5(1), 8(1), 16(2), 25(1)	0
2	27	1	15(1)	0
3	12	8	-	1
3	43	1	11(1)	0
4	5	1	-	0
4	34	3	14(1)	0
5	14	4	25(1)	1
5	30	2	15(1)	2
6	4	1	22(1)	0
6	22	9	-	1
7	11	1	5(1)	0
7	33	7	-	2
8	28	17	18(1)	3
9	29	9	3(1), 10(1)	2
9	51	4	-	0
10	1	1	1(1)	0
10	19	5	3(1)	2
11	23	5	21(1)	2
11	50	2	3(1), 17(1), 24(1)	1
12	2	1	-	0
12	41	1	-	2
13	7	4	-	1
13	18	1	-	0
13	48	2	-	0
14	15	1	24(1)	2
14	36	2	22(1)	1
14	40	2	-	0
15	21	1	20(1), 28(1), 11(1)	0
15	39	1	-	1

BmChr is the the most likely chromosome based on tblastx searches of LG-anchored spruce budworm loci against the *B. mori* integrated genome assembly; Hits indicates the number of unique *C. fumiferana* scaffolds with significant blast hits to the *B. mori* chromosome; Other Hits indicates the number of unique scaffolds (in parantheses) with significant hits to other chromosomes; UniRef90 indicates the number of blastx hits to known protein-coding genes in the UniRef90 protein database. *LG52 is designated the sex(Z) chromosome based entirely on the complete homozygosity in female progeny for paternally-informative alleles, but did not have significant blast hits to the *B. mori* Z chromosome.

Table 6-3: (continued)

BmChr	LG	Hits	Other Hits	UniRef90
17	9	3	-	1
17	35	1	-	2
18	13	5	6(1)	4
18	38	4	-	0
19	8	3	-	3
19	44	3	-	0
19	46	1	-	0
20	10	1	22(1)	1
20	25	4	18(1)	1
21	26	7	15(1)	5
22	17	4	3(1)	3
22	42	3	5(1), 11(1)	1
23	20	1	-	1
23	31	4	1(1)	2
23	49	2	28(1)	0
24	32	5	13(1)	0
25	16	3	-	0
25	47	3	-	0
26	37	3	-	0
27	45	2	1(1)	0
28	24	4	-	0
28	53	1	-	0
Unkn	3	-	-	0
Unkn	6	-	-	0

BmChr is the the most likely chromosome based on tblastx searches of LG-anchored spruce budworm loci against the *B. mori* integrated genome assembly; Hits indicates the number of unique *C. fumiferana* scaffolds with significant blast hits to the *B. mori* chromosome; Other Hits indicates the number of unique scaffolds (in parantheses) with significant hits to other chromosomes; UniRef90 indicates the number of blastx hits to known protein-coding genes in the UniRef90 protein database. *LG52 is designated the sex(Z) chromosome based entirely on the complete homozygosity in female progeny for paternally-informative alleles, but did not have significant blast hits to the *B. mori* Z chromosome.

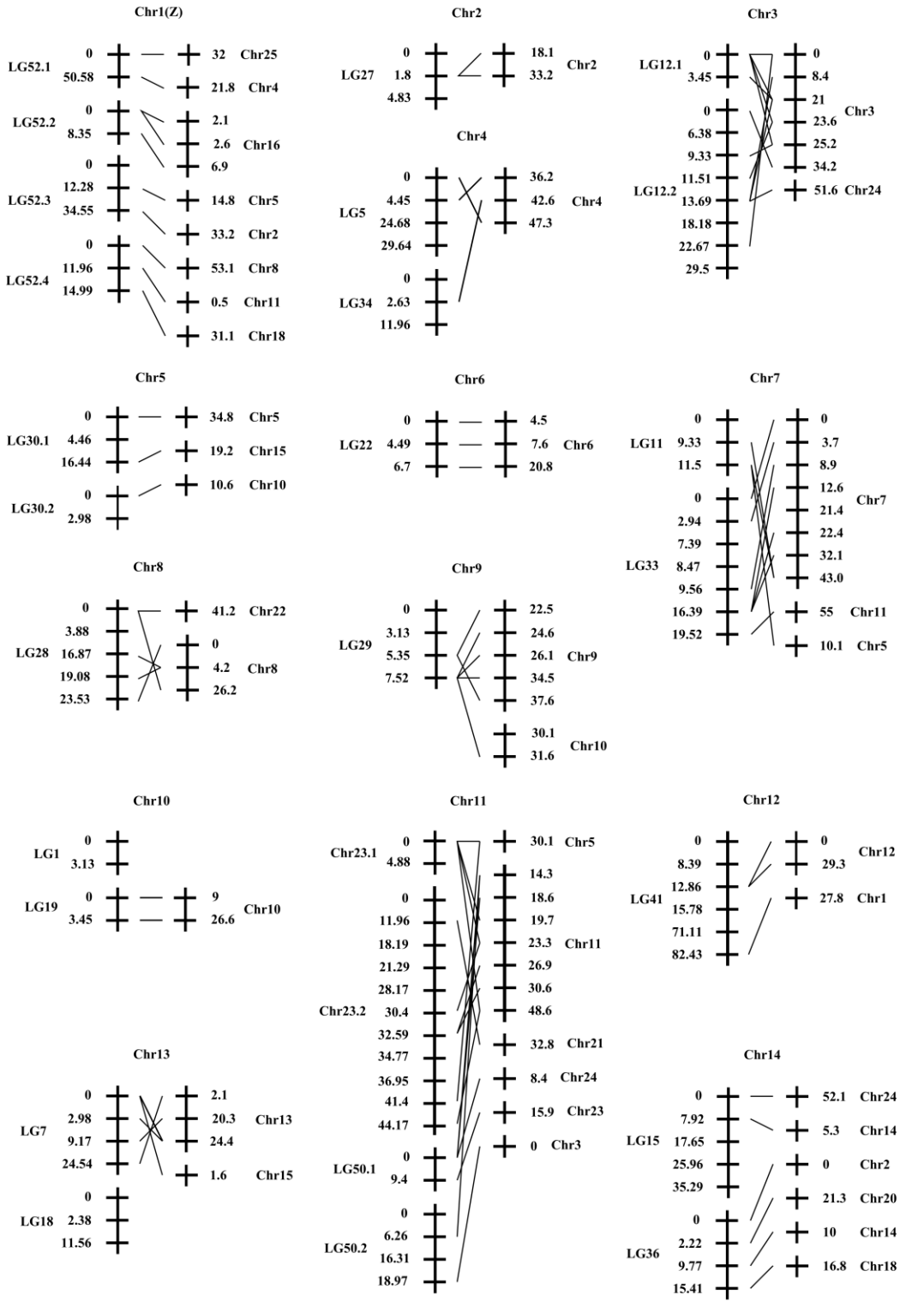


Figure 6-1: Spruce budworm linkage map indicating marker positions (cM) along each putative chromosome (left) and homologous regions within the *Bombyx mori* linkage map (right). Locus names at each position are listed in Appendix 6-1.

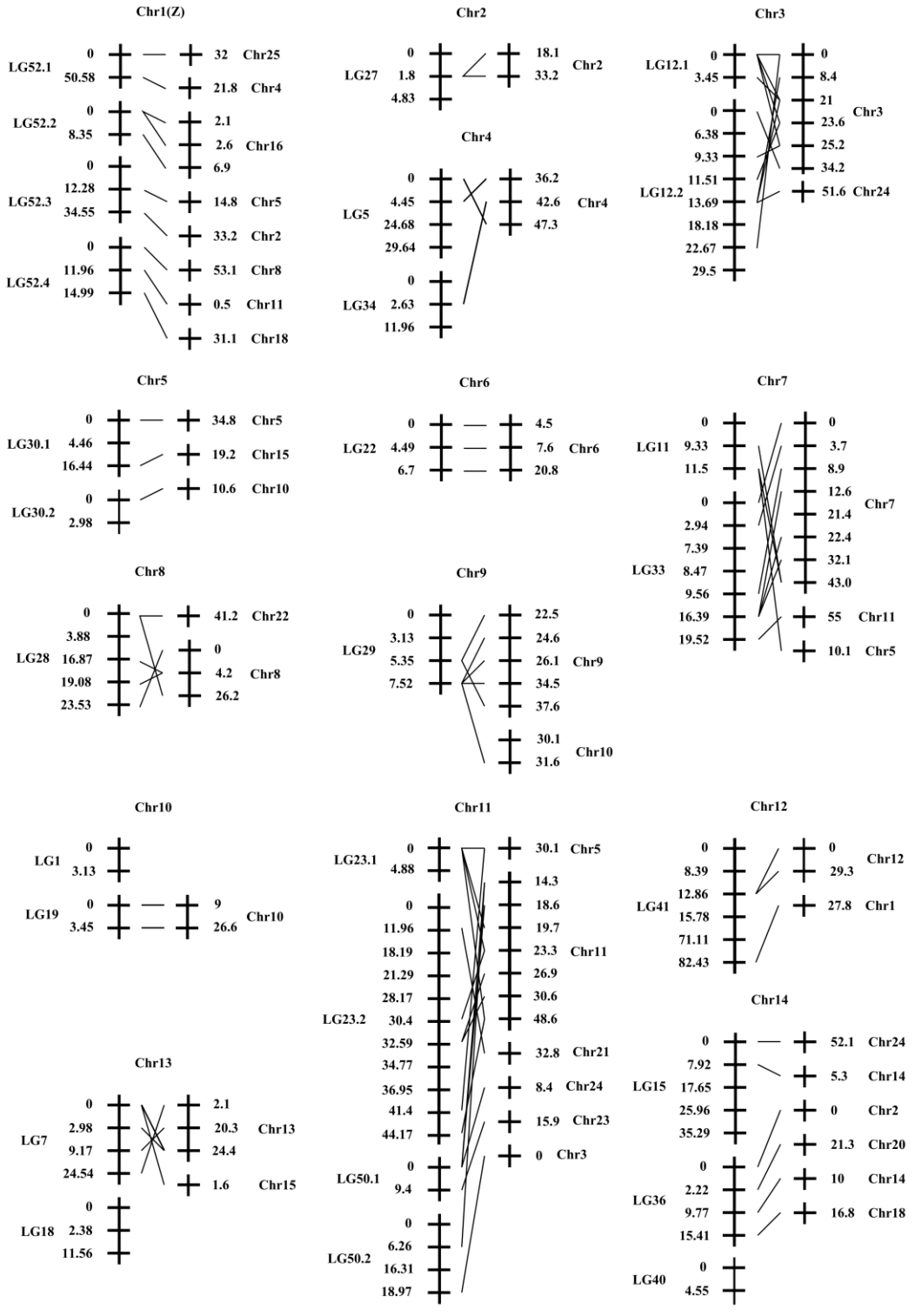


Figure 6-1: (continued)

Literature Cited

Andolfatto, P. Davison, D., Erezyilmaz, D., Hu, T.T., Mast, J., Sunayama-Moritaa, T., and D. Stern. 2011. Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome Research, 21: 610-617.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., and E.A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One, 3(10);e3376. Doi:10.1371/journal.pone.0003376

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., and M.L. Blaxter. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. PLoS One, 6(4): e19315. doi:10.1371/journal.pone.0019315

Beldade, P., Saenko, S.V., Pul, N., and A.D. Long. 2009. A gene-based linkage map for *Bicyclus anynana* butterflies allows for a comprehensive analysis of synteny with the lepidopteran reference genome. PLoS Genet 5(2): e1000366. Doi:10.1371/journal.pgen.1000366.

Bradshaw, Jr. H.D., Otto, K.G., Frewen, B.E., McKay, J.K., and D.W., Schemske. 1998. Quantitative trait loci affecting difference in floral morphology between two species of monkeyflower (*Mimulus*). Genetics, 149: 367-382.

Brunet, B.T.M., Doucet, D., Sturtevant, B.R., and F.A.H. Sperling. 2013. Characterization of EST-based SSR loci in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Conservation Genetic Resources, 5: 541-544.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., and J.H., Postlethwait. 2011. Stacks: Building and genotyping loci *De novo* from short-read sequences. Genes Genomes Genetics, 1: 171-182. Doi:10.1534/g3.111.000240.

Danecek, P., Auton, A., Abecasis, G., Albers, C., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics, 27: 2156-2158.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., and M.L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12: 499-510.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One, 6(5): e19379. Doi:10.1371/journal.pone.0019379.

Ennis, T.J. 1976. Sex chromatin and chromosome numbers in Lepidoptera. Canadian Journal of Genetics and Cytology, 18: 119-130.

Freeman, T.N. 1947. A new generic assignment for *Archips fumiferana* (Clemens), the spruce budworm (Lepidoptera, Tortricidae). The Canadian Entomologist, 79: 21-31.

Gadau, J., Page, Jr. R.E., J.H. Werren. 2002. Mapping of hybrid incompatibility loci in *Nasonia*. Genetics, 153: 1731-1741.

Gilligan, T.M., and J.W. Brown. 2014. A new name for the western spruce budworm (Lepidoptera: Tortricidae)? The Canadian Entomologist, 00: 1-7.

Groot, A.T., Staudacher, H., Barthel, A., Inglis, O., Schofl, G., Santangelo, R.G., Gebauer-jung, S., Vogel, H., Emerson, J., Schal, C., Heckel, D.G., and F. Gould. 2013. One quantitative trait locus for intra- and interspecific variation in a sex pheromone. Molecular Ecology, 22: 1065-1080.

Harvey, G.T. 1985a. The taxonomy of coniferophagous *Choristoneura* (Lepidoptera: Tortricidae): A review. Pp. 16-59 in C.J. Sanders, R.W. Stark, E.J. Mullins, And J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Heckel, D.G., Gahan, L.J., Liu, Y.-B., and B.E. Tabashnik. 1999. Genetic mapping of resistance to *Bacillus thuringiensis* toxins in diamondback moth using biphasic linkage analysis. Proceedings of the National Academy of Sciences of the United States of America, 96: 8373-8377.

Jiggins, C. D., Mavarez, J., Beltran, M., McMillan, W.O., Spencer, J.S., and E. Bermingham. 2005. A genetic linkage map of the mimetic Butterfly *Heliconius melpomene.* Genetics, 171: 557-570.

Kapan, D.D., Flanagan, N.S., Tobler, A., Papa, R., Reed, R.D., Acevedo Gonzalez, J., Ramirez Restreppo, M., Martinez, L., Maldonato, K., Ritschoff, C., Heckel, D.G., and W.O. McMillan. 2007. Localization of Mullerian mimicry genes on a dense linkage map of *Heliconius erato.* Genetics, 173: 735-757.

Lexer, C., Rosenthal, D.M., Raymond, O., Donovan, L.A., and L.H., Rieseberg. 2005. Genetics of species differences in the wild annual sunflowers *Helianthus annuus* and *H. petiolaris*. Genetics, 169: 2225-2239.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-1760.

Lukhtanov, V.A. 2000. Sex chromatin and sex chromosome systems in nonditrysian Lepidoptera (Insecta). Journal of Zoological Systematics and Evolutionary Research, 38: 73-79.

Lumley, L.M., Davis, C.S., and F.A.H. Sperling. 2009. Isolation and characterization of eight microsatellite loci in the spruce budworm species *Choristoneura fumiferana* and *Choristoneura occidentalis*, and cross-species amplification in related tortricid moths. Conservation Genetics Resources, 1: 501-504.

Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A., and E.A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research, 17: 240-248.

Nealis, V.G. 2005. Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist, 137: 584-597.

Nosil, P., Funk, D.J., and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology, 18: 375-402.

Pringle, E.G., Baxter, S.W., Webster, C.L., Papanicolaou, A., Lee, S.F., and C.D. Jiggins. 2007. Synteny and chromosome evolution in the Lepidoptera: Evidence from mapping in *Heliconius Melpomene*. Genetics, 177: 417-426.

Poland, J.A., Brown, P.J., Sorrells, M.E., and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLOS One 7:e32253. doi:10.1371/journal.pone.0032253.

Powell, J.A., and J.A. DeBenedictis. 1995. Evolutionary interpretations, taxonomy, and nomenclature. Pp. 217-275 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding Choristoneura (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Rastas, P., Paulin, L., Hanski, I., Lehtonen, R., and P. Auvinen. 2013. Lep-MAP: fast and accurate linkage map construction for large SNP datasets. Bioinformatics, 29: 3128-3134.

Razowski, J. 2008. Tortricidae (Lepidoptera) from South Africa. 6: Choristoneura Hubner and Procrica Diakonoff. Polish Journal of Entomology, 77: 245-254.

Sichova, J., Nyugen, P., Dalikova, M., and F. Marec. 2013. Chromosomal evolution in Tortricid moths: conserved karyotypes with diverged features. PLoS One, 8(5) e64520. Doi:10.1371/journal.pone.0064520.

Sinama, M., Dubut, V., Costeboat, C., Gilles, A., Junker, M., Malausa, T., Martin, J.-F., Neve, G., Pech, N., Schmitt, T., Zimmermann, M., and E., Meglecz. 2011. Challenges of microsatellite development in Lepidoptera: *Euphydras aurinia* (Nymphalidae) as a case study. European Jounral of Entomology, 108: 261-266.

Sonah, H., Bastien, M., Iquira, E., Tardive, A., Legare, G., Boyle, B., Normandeau, E., Laroche, J., Larose, S., Jean, M., and F. Belzile. 2013. An imporved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PLOS One 8:e54603. doi:10.1371/journal.pone.0054603

Suomalainen, E., Cook, L.M., and J.R.G. Turner. 1973. Achiasmatic oogenesis in the Heliconiine butterflies. Hereditas, 74: 302-304.

Strasburg, J.L., Sherman, N.A., Wright, K.M., Moyle, L.C., Willis, J.H., and L. Rieseberg. 2012. What can patterns of differentiation across plant genomes tell us about adaptation and speciation. Philosophical Transactions of the Royal Society B, 367: 364-373.

Tobler, A., Kapan, D., Flanagan, N.S., Gonzalez, C., Peterson,E., Jiggins, C.D., Johnston, S., Heckel, D.G., and W.O. McMillan. 2005. First generation linkage map of the warningly colored butterfly *Heliconius erato*. Heredity, 94: 408-417.

Turner, T.L., Hahn, M.W., and S.V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae*. PLoS Biology, 3:e285.

Van't Hof, A.E., Brakefield, P.M., Saccheri, I.J., and B.J. Zwaan. 2007. Evolutionary dynamics of multilocus microsatellite arrangements in the genome of the butterfly *Bicyclus anynana*, with implications for other Lepidoptera. Heredity, 98: 320-328.

Via, S., and J. West. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Molecular Ecology, 17: 4334-4345.

Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B, 367: 451-460.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (Choristoneura spp.) biotype reactions to forest and climate characterisitics. Global Change Biology, 13: 1630-1643.

Wang, B., and A.H. Porter. 2004. An AFLP-based interspecific linkage map of sympatric hybridizing *Colias* butterflies. Genetics, 168: 215-225.

Yammamoto, K., Nohata, J., Kadono-Okuda, K., Narukawa, J., Sasanuma, M., Sasanuma, S., Minami, H., Shimomura, M., Suetsugo, Y., Banno, Y., Osoegawa, K., de Jong, P.J., Goldsmith, M.R., and K. Mita. 2008. A BAC-based integrated linkage map of the silkworm *Bombyx mori*. Genome Biology, 9: R21. Doi:10.1186/gb-2008-9-1-r21.

Yasukochi, Y., Ashakumary, L.A., Baba, K., Yoshida, A., Sahara, K. 2006. A second generation integrated map of the silkworm reveals synteny and conserved gene order between lepidopteran insects. Genetics, 173: 1319-1328.

Zhang, D.-X. 2004. Lepidopteran microsatellite DNA: redundant but promising. Trends in Ecology and Evolution, 19: 507-509.

Zhulidov, P.A., Bogdanova, E.A., Shcheglov, A.S., Vagner, L.L., Khaspekov, G.L., Kozhemyako, V.B., Matz, M.V., Meleshkevitch, E., Moroz, L.L., Lukyanov, S.A., and D.A. Shagin. 2004. Simple cDNA normalization using kamchatka crab duplex-specific nuclease. Nucleic Acids Research 32:e37. doi:10.1093/nar/gnh031.

Appendix 6-1: Locus-specific linkage mapping characterisitics, and *Bombyx mori* orthologs.

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
2	9226	4	0	4	0	0	0	na	na	Na
2	198215	0	20	4	0	0	0	na	na	Na
2	198230	0	20	4	0	0	0	na	na	na
2	198242	4	0	4	0	0	0	na	na	na
2	300820	0	20	4	0	0	0	na	na	na
2	300832	0	20	4	0	0	0	na	na	na
3	83459	30	0	30	0	0	0	na	na	na
5	148778	0	27	0	0	0	0	na	na	na
5	243950	15	0	15	0	0	14	14	na	na
5	243952	15	0	15	0	0	14	14	na	na
5	244000	15	0	15	0	0	14	14	na	na
5	244017	6	0	0	0	0	0	na	na	na
6	221832	38	0	38	0	0	0	na	na	na
7	70926	0	36	25	25	25.64	0	na	na	na
7	70936	0	36	25	25	25.64	0	na	na	na
7	86930	0	36	25	25	25.64	0	na	na	na
7	127073	0	36	25	25	25.64	0	na	na	na
7	127098	0	36	25	25	25.64	0	na	na	na
9	170974	0	3	0	0	0	0	na	na	na
10	103904	0	51	0	0	0	0	na	na	na
11	213602	0	6	0	0	0	0	na	na	na
14	166571	18	0	18	0	0	0	na	na	na
14	166641	18	0	18	0	0	0	na	na	na
15	125619	23	0	0	0	0	0	na	na	na
17	62543	0	8	28	0	0	8	8	na	na
19	136865	4	0	0	0	0	0	na	na	na
21	7637	0	13	38	0	0	18	18	na	na
21	7686	38	0	38	0	0	18	18	na	na
21	7690	38	0	38	0	0	18	18	na	na
23	98809	0	7	0	0	0	0	na	na	na
23	98813	14	0	0	0	0	0	na	na	na
23	98933	14	0	0	0	0	0	na	na	na
24	18986	11	0	0	0	0	0	na	na	na
27	21670	13	0	0	0	0	0	na	na	na
27	21682	0	13	0	0	0	0	na	na	na
27	21754	0	46	0	0	0	0	na	na	na
28	122156	8	0	0	0	0	0	na	na	na
29	67321	0	6	0	0	0	0	na	na	na
30	16672	0	20	0	0	0	0	na	na	na
31	64001	3	0	0	0	0	0	na	na	na
31	68239	0	11	0	0	0	0	na	na	na
31	68248	16	0	0	0	0	0	na	na	na
31	89909	3	0	0	0	0	0	na	na	na
32	47461	43	0	0	0	0	0	na	na	na
35	12504	0	12	26	0	0	0	na	na	na
35	12552	0	12	26	0	0	0	na	na	na
35	26958	0	12	26	0	0	0	21	na	na
35	61442	26	0	26	0	0	21	21	na	na
35	61530	26	0	26	0	0	21	21	na	na
36	14664	0	1	0	0	0	0	na	na	na
36	14670	0	1	0	0	0	0	na	na	na
36	14704	0	1	0	0	0	0	na	na	na
36	14706	0	1	0	0	0	0	na	na	na
36	14720	0	1	0	0	0	0	na	na	na
36	116828	0	1	0	0	0	0	na	na	na
36	116845	0	1	0	0	0	0	na	na	na
36	128245	0	18	0	0	0	0	na	na	na
36	128331	1	0	0	0	0	0	na	na	na
36	128341	0	18	0	0	0	0	na	na	na
36	128356	0	18	0	0	0	0	na	na	na
36	128358	0	1	0	0	0	0	na	na	na
41	33589	0	12	0	0	0	0	na	na	na
42	66018	0	57	0	0	0	0	na	na	na
43	109427	0	26	23	23	0	0	5	T032J18	30.1cM
43	109452	0	19	23	23.2	41.4	0	5	T032J18	30.1cM
43	109462	0	19	23	23.2	41.4	0	5	T032J18	30.1cM
44	43660	0	7	0	0	0	0	na	na	na
44	124213	0	3	0	0	0	0	na	na	na
44	124227	0	3	0	0	0	0	na	na	na
45	1566	16	0	0	0	0	0	na	na	na
45	98147	0	21	36	36	15.41	0	18	T050B23	16.8cM
47	1387	13	0	13	0	0	18	18	na	na
47	41640	0	46	13	13	4.45	18	18	T639I03	22.2cM
47	41655	0	46	13	13	4.45	18	18	T639I03	22.2cM
48	91492	17	0	17	0	0	0	10	na	na
48	91558	17	0	17	0	0	0	10	na	na
49	44932	0	7	0	0	0	0	na	na	na
51	25462	20	0	20	0	0	0	na	na	na
51	55498	20	0	20	0	0	0	23	na	na
52	93912	0	6	35	35	0	0	7	T048E15	22.4cM

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
52	93924	0	6	35	35	0	0	7	T048E15	22.4cM
52	93995	0	6	35	35	0	0	7	T048E15	22.4cM
52	108645	0	6	35	35	5.27	0	24	T602I07	51.6cM
53	91095	30	0	30	0	0	5	5	na	na
53	114919	0	7	30	30	0	5	5	T044D05	34.8cM
53	114922	0	7	30	30	0	5	5	T044D05	34.8cM
54	54762	0	3	0	0	0	0	na	na	na
55	4734	0	4	0	0	0	0	na	na	na
55	4738	7	0	0	0	0	0	na	na	na
55	110652	7	0	0	0	0	0	na	na	na
56	14890	0	36	25	25	25.64	0	na	na	na
56	14916	0	36	25	25	25.64	0	na	na	na
56	129718	25	0	25	0	0	0	1	na	na
56	129771	25	0	25	0	0	0	1	na	na
60	93605	0	10	0	0	0	0	na	na	na
61	22237	49	0	49	0	0	0	na	na	na
61	22300	49	0	49	0	0	0	na	na	na
61	22308	49	0	49	0	0	0	na	na	na
64	110466	0	7	0	0	0	0	na	na	na
65	46355	9	0	9	0	0	0	na	na	na
65	46373	9	0	9	0	0	0	na	na	na
65	57854	0	6	9	0	0	0	na	na	na
65	57855	0	6	9	0	0	0	na	na	na
65	57917	0	6	9	0	0	0	na	na	na
65	57998	0	31	9	9	4.45	0	na	na	na
65	58013	9	0	9	0	0	0	na	na	na
65	100944	0	31	9	9	4.45	0	na	na	na
66	4082	14	0	0	0	0	0	na	na	na
67	83310	14	0	0	0	0	0	na	na	na
70	81848	0	1	0	0	0	0	na	na	na
70	109162	0	1	0	0	0	0	na	na	na
72	25535	0	3	0	0	0	0	na	na	na
77	2025	0	14	0	0	0	0	na	na	na
78	13525	2	0	0	0	0	0	na	na	na
78	51258	0	9	0	0	0	0	na	na	na
78	62475	2	0	0	0	0	0	na	na	na
78	73567	0	9	0	0	0	0	na	na	na
79	40195	0	7	0	0	0	0	na	na	na
81	81930	0	57	0	0	0	0	na	na	na
84	69471	8	0	0	0	0	0	na	na	na
84	69485	0	25	0	0	0	0	na	na	na
84	69500	8	0	0	0	0	0	na	na	na
85	23049	0	57	0	0	0	0	na	na	na
86	75002	0	3	14	0	0	5	5	na	na
86	75023	0	3	14	0	0	5	5	na	na
86	75077	0	3	14	0	0	5	5	na	na
87	43633	0	3	0	0	0	0	na	na	na
93	41322	0	45	42	42	16.27	0	na	na	na
93	119037	0	28	42	0	0	22	22	na	na
94	7488	0	42	0	0	0	0	na	na	na
96	21792	0	1	0	0	0	0	na	na	na
96	21859	0	1	0	0	0	0	na	na	na
96	54790	0	1	0	0	0	0	na	na	na
97	53150	0	19	23	23.2	30.4	11	11	T618J01	23.3cM
97	53179	0	19	23	23.2	30.4	11	11	T618J01	23.3cM
99	54408	0	9	0	0	0	0	na	na	na
100	21370	1	0	0	0	0	0	na	na	na
100	21449	0	1	0	0	0	0	na	na	na
101	291	0	14	0	0	0	0	na	na	na
108	21740	35	0	0	0	0	0	na	na	na
109	2087	12	0	0	0	0	0	na	na	na
110	51567	0	34	8	8	12.12	0	na	na	na
110	51608	0	34	8	8	12.12	0	na	na	na
110	114431	8	0	8	0	0	0	19	na	na
114	38491	1	0	1	0	0	0	na	na	na
114	38514	1	0	1	0	0	0	na	na	na
114	38524	1	0	1	0	0	0	na	na	na
114	38544	1	0	1	0	0	0	na	na	na
119	69003	0	42	0	0	0	0	na	na	na
119	103680	0	42	0	0	0	0	na	na	na
121	15846	0	31	9	9	4.45	0	na	na	na
121	15865	0	31	9	9	4.45	0	na	na	na
122	6211	2	0	0	0	0	0	na	na	na
124	3840	13	0	0	0	0	0	na	na	na
127	67329	0	51	0	0	0	0	na	na	na
133	24433	7	0	7	0	0	13	13	na	na
133	24463	7	0	7	0	0	13	13	na	na
133	24466	18	0	7	0	0	13	13	na	na
133	24473	7	0	7	0	0	13	13	na	na
133	24483	7	0	7	0	0	13	13	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
133	24486	7	0	7	0	0	13	13	na	na
134	50039	18	0	0	0	0	0	na	na	na
136	83335	6	0	0	0	0	0	na	na	na
136	83390	6	0	0	0	0	0	na	na	na
136	85829	6	0	0	0	0	0	na	na	na
138	3027	6	0	0	0	0	0	na	na	na
139	34840	15	0	15	0	0	24	24	na	na
139	34846	0	43	15	0	0	24	24	na	na
139	34901	0	43	15	0	0	24	24	na	na
140	14224	0	31	50	0	0	17	17	na	na
140	14263	50	0	50	0	0	17	17	na	na
143	22035	0	34	8	8	14.3	0	na	na	na
143	22041	8	0	8	0	0	0	na	na	na
143	46893	8	0	8	0	0	0	25	na	na
143	94372	8	0	8	0	0	0	na	na	na
150	77877	24	0	24	0	0	0	na	na	na
151	4158	2	0	0	0	0	0	na	na	na
152	33335	0	32	49	0	0	28	28	na	na
152	33360	49	0	49	0	0	28	28	na	na
155	32007	0	57	0	0	0	0	na	na	na
156	148	0	18	6	0	0	0	na	na	na
156	71854	6	0	6	0	0	0	na	na	na
156	71863	6	0	6	0	0	0	na	na	na
158	26351	3	0	0	0	0	0	na	na	na
159	50179	26	0	26	0	0	21	21	na	na
162	61579	41	0	41	0	0	0	na	na	na
162	61637	0	9	41	41	71.11	0	na	na	na
163	24637	22	0	22	0	0	0	na	na	na
163	24692	4	0	22	0	0	0	na	na	na
164	49936	0	42	31	0	0	1	1	na	na
164	49969	0	42	31	0	0	1	1	na	na
164	75962	31	0	31	0	0	0	3	na	na
164	75979	0	14	31	31	51.5	0	3	S096A04	4.2cM
164	76029	0	14	31	31	51.5	0	3	S096A04	4.2cM
164	92998	0	52	31	0	0	0	na	na	na
168	16787	1	0	19	0	0	10	10	na	na
168	30694	1	0	19	0	0	0	na	na	na
168	61278	19	0	19	0	0	10	10	na	na
170	60868	0	19	23	23.2	34.77	0	na	na	na
170	77498	43	0	23	0	0	0	27	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
172	991	0	29	22	22	4.49	6	6	T604M13	7.6cM
172	994	0	29	22	22	4.49	6	6	T604M13	7.6cM
172	1059	0	20	22	0	0	6	6	na	na
176	79794	0	2	0	0	0	0	na	na	na
181	67297	2	0	0	0	0	0	na	na	na
181	67302	2	0	0	0	0	0	na	na	na
181	67308	2	0	0	0	0	0	na	na	na
181	67376	0	9	0	0	0	0	na	na	na
181	67410	0	23	0	0	0	0	na	na	na
182	1901	0	26	0	0	0	0	na	na	na
188	16191	50	0	50	0	0	0	na	na	na
188	16239	50	0	50	0	0	0	na	na	na
188	16240	50	0	50	0	0	0	na	na	na
188	46986	50	0	50	0	0	0	na	na	na
188	47014	50	0	50	0	0	0	na	na	na
189	49974	0	13	38	0	0	0	na	na	na
189	55111	38	0	38	0	0	18	18	na	na
189	81747	13	0	38	0	0	0	na	na	na
192	68677	18	0	0	0	0	0	na	na	na
192	68691	0	10	0	0	0	0	na	na	na
196	70444	0	64	24	24	27.76	0	na	na	na
200	37138	0	16	12	12.2	18.18	0	na	na	na
200	37148	0	17	12	12.1	0	0	na	na	na
202	68357	31	0	31	0	0	0	na	na	na
202	83905	20	0	31	0	0	23	23	na	na
215	33682	7	0	0	0	0	0	na	na	na
215	33721	7	0	0	0	0	0	na	na	na
217	5446	41	0	41	0	0	0	12	na	na
217	28473	2	0	41	0	0	12	12	na	na
217	28535	0	23	41	41	12.86	12	12	T029N20	29.3cM
217	28537	0	23	41	41	12.86	12	12	T029N20	29.3cM
217	39920	0	23	41	41	12.86	0	na	na	na
217	68948	0	23	41	41	12.86	12	12	S006F19	0.0cM
217	69031	0	23	41	41	12.86	12	12	S006F19	0.0cM
221	59030	0	16	0	0	0	0	na	na	na
226	28078	0	51	28	28	0	0	22	T077L13	41.2cM
226	28119	0	8	28	0	0	0	22	na	na
226	28137	0	8	28	0	0	0	22	na	na
232	57456	0	16	12	12.2	22.67	3	3	S601M01	0.0cM
232	57475	0	17	12	12.1	0	3	3	S601M01	0.0cM

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
232	57482	0	16	12	12.2	22.67	3	3	S601M01	0.0cM
232	57487	0	17	12	12.1	0	3	3	S601M01	0.0cM
232	57517	0	17	12	12.1	0	3	3	S601M01	0.0cM
232	57518	12	0	12	0	0	3	3	na	na
232	57538	0	17	12	12.1	0	3	3	S601M01	0.0cM
243	12865	0	13	0	0	0	0	na	na	na
247	24007	7	0	7	0	0	13	13	na	na
247	24109	0	10	7	7	0	13	13	T047A11	24.4cM
247	76145	0	10	7	7	2.98	13	13	T047A11	24.4cM
247	76168	0	10	7	7	2.98	13	13	T047A11	24.4cM
257	39897	0	6	0	0	0	0	na	na	na
260	79707	0	7	0	0	0	0	na	na	na
262	32246	0	2	0	0	0	0	na	na	na
263	57256	0	2	0	0	0	0	na	na	na
267	73738	0	26	23	23	0	0	na	na	na
267	73747	0	26	23	23	0	0	na	na	na
267	73756	0	26	23	23	0	0	na	na	na
267	73796	0	26	23	23	0	0	na	na	na
268	31307	13	0	0	0	0	0	na	na	na
268	56838	13	0	0	0	0	0	na	na	na
268	56911	0	13	0	0	0	0	na	na	na
268	56938	0	13	0	0	0	0	na	na	na
270	3397	0	26	23	23	4.88	0	na	na	na
270	3402	23	0	23	0	0	0	na	na	na
270	80337	0	27	0	0	0	0	na	na	na
271	68549	11	0	0	0	0	0	na	na	na
271	68552	11	0	0	0	0	0	na	na	na
279	60247	24	0	24	0	0	0	na	na	na
279	60249	0	32	24	0	0	0	na	na	na
279	60273	0	32	24	0	0	0	na	na	na
280	39151	0	30	0	0	0	0	na	na	na
280	39155	0	37	0	0	0	0	na	na	na
280	70774	0	30	0	0	0	0	na	na	na
280	70807	0	30	0	0	0	0	na	na	na
283	5896	29	0	29	0	0	0	na	na	na
283	5933	29	0	29	0	0	0	na	na	na
283	5950	29	0	29	0	0	0	na	na	na
283	73984	0	16	29	0	0	3	3	na	na
288	28362	0	5	0	0	0	0	na	na	na
291	78692	19	0	19	0	0	0	na	na	na
298	24581	0	24	0	0	0	0	na	na	na
309	11390	0	37	29	29	7.52	0	na	na	na
309	31041	29	0	29	0	0	9	9	na	na
313	32618	42	0	42	0	0	0	na	na	na
314	15708	0	13	0	0	0	0	na	na	na
314	15711	13	0	0	0	0	0	na	na	na
315	8218	1	0	0	0	0	0	na	na	na
318	47617	26	0	26	0	0	0	15	na	na
319	52677	9	0	9	0	0	0	na	na	na
319	57362	0	31	9	9	0	17	17	T619N03	0.0cM
320	73650	0	2	0	0	0	0	na	na	na
320	73688	5	0	0	0	0	0	na	na	na
328	6856	19	0	19	0	0	0	na	na	na
328	6865	19	0	19	0	0	0	na	na	na
331	58171	5	0	5	0	0	0	na	na	na
331	58249	5	0	5	0	0	0	na	na	na
331	58250	5	0	5	0	0	0	na	na	na
331	58251	5	0	5	0	0	0	na	na	na
333	3501	14	0	0	0	0	0	na	na	na
333	3554	0	7	0	0	0	0	na	na	na
333	61589	14	0	0	0	0	0	na	na	na
336	29733	0	1	19	19	3.45	0	10	T085D18	9.0cM
336	29740	19	0	19	0	0	0	10	na	na
339	11496	18	0	0	0	0	0	na	na	na
344	5873	13	0	13	0	0	18	18	na	na
344	5878	13	0	13	0	0	18	18	na	na
344	18410	13	0	13	0	0	18	18	na	na
344	24833	0	46	13	13	0	0	na	na	na
344	24841	13	0	13	0	0	0	na	na	na
348	31198	0	1	0	0	0	0	na	na	na
348	31280	0	1	0	0	0	0	na	na	na
348	62662	1	0	0	0	0	0	na	na	na
350	48403	22	0	22	0	0	6	6	na	na
351	41474	0	12	0	0	0	0	na	na	na
359	53783	0	23	0	0	0	0	na	na	na
361	9593	3	0	0	0	0	0	na	na	na
361	42412	3	0	0	0	0	0	na	na	na
374	39983	0	46	0	0	0	0	na	na	na
375	50354	5	0	0	0	0	0	na	na	na
378	64337	0	57	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
384	64556	0	14	0	0	0	0	na	na	na
398	40572	0	29	0	0	0	0	na	na	na
398	40623	0	20	0	0	0	0	na	na	na
404	7127	0	51	28	28	3.88	0	na	na	na
404	7170	0	8	28	0	0	0	na	na	na
404	44725	28	0	28	0	0	0	na	na	na
407	61942	0	46	0	0	0	0	na	na	na
411	39917	26	0	26	0	0	21	21	na	na
413	51390	7	0	0	0	0	0	na	na	na
417	23803	0	51	28	28	0	0	8	T639D05	26.2cM
417	61298	0	8	28	0	0	18	18	na	na
419	32321	22	0	22	0	0	6	6	na	na
419	32354	22	0	22	0	0	6	6	na	na
427	36847	8	0	0	0	0	0	na	na	na
439	63904	0	17	12	12.1	0	0	na	na	na
439	63934	12	0	12	0	0	0	na	na	na
452	14650	0	52	0	0	0	0	na	na	na
453	21237	0	3	14	0	0	0	na	na	na
453	57879	0	3	14	0	0	0	na	na	na
453	57941	0	3	14	0	0	0	na	na	na
472	53334	0	33	33	33	16.39	7	7	T002O23	32.1cM
472	53367	0	35	33	0	0	7	7	na	na
472	54957	0	33	33	33	16.39	0	na	na	na
475	48960	0	15	10	0	0	0	5	na	na
475	48966	10	0	10	0	0	0	5	na	na
475	49011	0	15	10	0	0	0	5	na	na
478	25834	43	0	43	0	0	0	na	na	na
478	25893	0	22	43	0	0	0	na	na	na
482	42008	40	0	40	0	0	0	na	na	na
487	6947	19	0	19	0	0	10	10	na	na
487	6964	19	0	19	0	0	10	10	na	na
494	57054	0	4	0	0	0	0	na	na	na
501	14613	0	3	0	0	0	0	na	na	na
506	24192	0	20	0	0	0	0	na	na	na
508	31643	0	16	12	12.2	9.33	0	na	na	na
508	31691	0	16	12	12.2	9.33	0	na	na	na
509	2917	0	63	0	0	0	0	na	na	na
510	31967	14	0	14	0	0	0	na	na	na
510	31968	0	3	14	0	0	0	na	na	na
510	32002	0	3	14	0	0	0	na	na	na
510	32804	0	7	14	0	0	5	5	na	na
514	10226	17	0	17	0	0	22	22	na	na
524	51605	5	0	0	0	0	0	na	na	na
524	51652	5	0	0	0	0	0	na	na	na
524	51662	5	0	0	0	0	0	na	na	na
528	39043	0	51	0	0	0	0	na	na	na
533	56262	0	9	0	0	0	0	na	na	na
533	56268	0	9	0	0	0	0	na	na	na
533	56282	0	9	0	0	0	0	na	na	na
534	50342	0	39	26	26	9.33	21	21	T628C17	26.6cM
534	50395	0	39	26	26	9.33	21	21	T628C17	26.6cM
534	50408	0	39	26	26	9.33	21	21	T628C17	26.6cM
535	36275	2	0	0	0	0	0	na	na	na
538	3814	14	0	39	0	0	15	15	na	na
538	28277	39	0	39	0	0	0	na	na	na
539	19909	23	0	0	0	0	0	na	na	na
552	27198	1	0	0	0	0	0	na	na	na
552	27411	1	0	0	0	0	0	na	na	na
553	8899	10	0	0	0	0	0	na	na	na
573	32717	0	71	50	0	0	0	na	na	na
573	32747	0	71	50	0	0	0	na	na	na
574	4641	0	14	0	0	0	0	na	na	na
580	57976	2	0	0	0	0	0	na	na	na
580	58018	2	0	0	0	0	0	na	na	na
581	18583	30	0	30	0	0	0	21	na	na
583	11115	12	0	0	0	0	0	na	na	na
600	41441	7	0	0	0	0	0	na	na	na
607	47840	0	22	0	0	0	0	na	na	na
609	40651	11	0	0	0	0	0	na	na	na
610	37108	27	0	0	0	0	0	na	na	na
614	2046	0	31	9	9	4.45	0	na	na	na
614	2060	0	31	9	9	4.45	0	na	na	na
616	755	0	18	0	0	0	0	na	na	na
619	16776	19	0	19	0	0	0	na	na	na
619	16828	0	1	19	19	0	0	na	na	na
626	3109	0	6	9	0	0	17	17	na	na
626	3128	0	31	9	9	23.65	17	17	S654P10	16.4cM
626	3160	0	6	9	0	0	17	17	na	na
630	358	42	0	42	0	0	0	na	na	na
631	55944	0	10	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
640	53574	0	11	0	0	0	0	na	na	na
646	54244	4	0	0	0	0	0	na	na	na
658	46942	6	0	36	0	0	0	na	na	na
658	46950	36	0	36	0	0	0	na	na	na
664	29091	30	0	30	0	0	0	na	na	na
671	15277	42	0	42	0	0	0	na	na	na
676	49330	0	13	0	0	0	0	na	na	na
676	49335	13	0	0	0	0	0	na	na	na
677	46826	0	24	16	16	9.71	25	25	B009K19	28.8cM
677	46962	0	24	16	16	9.71	25	25	B009K19	28.8cM
677	46974	0	11	16	0	0	25	25	na	na
679	6654	0	40	0	0	0	0	na	na	na
682	47691	51	0	51	0	0	0	na	na	na
682	53148	0	30	51	0	0	9	9	na	na
683	22492	25	0	25	0	0	20	20	na	na
683	53075	0	36	25	25	16.31	0	na	na	na
689	41082	40	0	40	0	0	0	na	na	na
689	41122	6	0	40	0	0	0	na	na	na
689	47817	0	21	40	40	0	0	na	na	na
694	39767	0	4	0	0	0	0	na	na	na
696	21362	14	0	0	0	0	0	na	na	na
696	21382	0	7	0	0	0	0	na	na	na
697	18019	0	24	0	0	0	0	na	na	na
697	18024	0	11	0	0	0	0	na	na	na
697	18031	0	11	0	0	0	0	na	na	na
697	43415	3	0	0	0	0	0	na	na	na
699	14038	0	3	14	0	0	5	5	na	na
699	14082	0	3	14	0	0	5	5	na	na
699	14095	0	3	14	0	0	5	5	na	na
707	888	0	4	0	0	0	0	na	na	na
714	40715	0	25	0	0	0	0	na	na	na
722	11393	29	0	29	0	0	0	na	na	na
726	26612	0	45	42	42	16.27	0	na	na	na
729	11401	0	26	0	0	0	0	na	na	na
738	18354	2	0	0	0	0	0	na	na	na
739	51219	28	0	28	0	0	8	8	na	na
745	46044	0	18	0	0	0	0	na	na	na
745	46071	0	18	0	0	0	0	na	na	na
750	33332	16	0	0	0	0	0	na	na	na
751	47347	0	34	8	8	14.3	0	13	T613H22	33.3cM

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
761	44954	51	0	51	0	0	9	9	na	na
778	45442	36	0	36	0	0	0	na	na	na
778	45454	36	0	36	0	0	0	na	na	na
780	35804	42	0	42	0	0	0	na	na	na
782	15743	4	0	0	0	0	0	na	na	na
782	15756	4	0	0	0	0	0	na	na	na
784	46609	24	0	24	0	0	0	28	na	na
784	46680	0	64	24	24	27.76	0	28	T636K06	43.4cM
785	6948	0	14	0	0	0	0	na	na	na
792	19332	0	10	0	0	0	0	na	na	na
795	40310	0	5	0	0	0	0	na	na	na
795	40387	0	2	0	0	0	0	na	na	na
801	48443	39	0	0	0	0	0	na	na	na
805	44139	31	0	31	0	0	0	na	na	na
808	788	27	0	0	0	0	0	na	na	na
809	10911	0	45	42	42	16.27	0	na	na	na
812	24815	0	15	0	0	0	0	na	na	na
813	5484	0	15	0	0	0	0	na	na	na
814	27095	0	16	12	12.2	6.38	0	na	na	na
814	40483	0	17	12	12.1	0	0	na	na	na
833	12044	0	28	0	0	0	0	na	na	na
858	3050	27	0	0	0	0	0	na	na	na
873	8424	14	0	0	0	0	0	na	na	na
875	8170	0	30	29	0	0	9	9	na	na
875	29973	0	37	29	29	7.52	0	na	na	na
875	29996	0	30	29	0	0	0	na	na	na
875	30004	0	30	29	0	0	0	na	na	na
875	30034	29	0	29	0	0	0	na	na	na
879	27552	0	32	0	0	0	0	na	na	na
882	7495	15	0	15	0	0	0	na	na	na
882	7496	15	0	15	0	0	0	na	na	na
882	28316	0	63	15	15	0	0	24	S002L07	52.1cM
886	8654	21	0	21	0	0	15	15	na	na
886	8675	21	0	21	0	0	15	15	na	na
892	29546	13	0	38	0	0	18	18	na	na
892	30000	0	13	38	0	0	18	18	na	na
892	30012	0	13	38	0	0	18	18	na	na
892	30031	0	13	38	0	0	18	18	na	na
893	5133	0	37	29	29	5.35	9	9	T057A16	37.6cM
893	32220	0	37	29	29	5.35	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
901	25177	20	0	0	0	0	0	na	na	na
908	33609	0	26	23	23	0	11	11	T618J01	23.3cM
908	33619	0	26	23	23	0	11	11	T618J01	23.3cM
910	17618	5	0	0	0	0	0	na	na	na
910	17652	5	0	0	0	0	0	na	na	na
910	36113	0	2	0	0	0	0	na	na	na
912	30549	42	0	42	0	0	0	na	na	na
912	30563	42	0	42	0	0	0	na	na	na
912	30591	0	45	42	42	16.27	0	na	na	na
935	6033	0	11	0	0	0	0	na	na	na
935	12193	16	0	0	0	0	0	na	na	na
955	41917	0	19	23	23.2	28.17	0	na	na	na
960	513	0	14	0	0	0	0	na	na	na
960	584	0	14	0	0	0	0	na	na	na
990	8848	0	10	0	0	0	0	na	na	na
990	20157	0	4	0	0	0	0	na	na	na
992	25919	3	0	0	0	0	0	na	na	na
995	43534	0	3	0	0	0	0	na	na	na
1001	42798	4	0	0	0	0	0	na	na	na
1001	42868	4	0	0	0	0	0	na	na	na
1002	6630	19	0	19	0	0	10	10	na	na
1012	14604	0	45	42	42	16.27	5	5	B551F24	25.4cM
1013	6545	0	34	8	8	16.47	0	19	T075C02	25.9cM
1013	6572	0	34	8	8	16.47	0	19	T075C02	25.9cM
1015	31767	21	0	0	0	0	0	na	na	na
1015	31783	28	0	0	0	0	0	na	na	na
1020	21169	0	39	0	0	0	0	na	na	na
1024	27473	0	65	21	21	4.56	0	16	T038C06	50.3cM
1024	32943	0	65	21	21	4.56	0	16	T038C06	50.3cM
1025	10601	0	25	8	0	0	0	na	na	na
1025	10715	0	34	8	8	14.3	0	na	na	na
1038	783	0	24	0	0	0	0	na	na	na
1039	28342	35	0	0	0	0	0	na	na	na
1040	38991	47	0	47	0	0	0	na	na	na
1049	37531	0	18	0	0	0	0	na	na	na
1049	41101	0	18	0	0	0	0	na	na	na
1057	23499	0	5	0	0	0	0	na	na	na
1057	23551	0	2	0	0	0	0	na	na	na
1060	7551	26	0	26	0	0	0	na	na	na
1060	7581	26	0	26	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
1060	7582	26	0	26	0	0	0	na	na	na
1063	15869	8	0	0	0	0	0	na	na	na
1073	12583	0	3	0	0	0	0	na	na	na
1081	41855	26	0	26	0	0	0	na	na	na
1088	23133	0	9	0	0	0	0	na	na	na
1088	23135	0	23	0	0	0	0	na	na	na
1089	5224	8	0	8	0	0	0	na	na	na
1089	5244	0	34	8	8	14.3	0	na	na	na
1097	28483	0	31	0	0	0	0	na	na	na
1112	33556	14	0	0	0	0	0	na	na	na
1121	5083	0	24	0	0	0	0	na	na	na
1121	5120	3	0	0	0	0	0	na	na	na
1121	5121	3	0	0	0	0	0	na	na	na
1121	5123	3	0	0	0	0	0	na	na	na
1133	717	18	0	0	0	0	0	na	na	na
1140	34021	29	0	29	0	0	0	na	na	na
1143	11940	12	0	49	0	0	0	1	na	na
1143	31477	49	0	49	0	0	0	na	na	na
1148	17465	12	0	12	0	0	0	3	na	na
1148	18827	12	0	12	0	0	3	3	na	na
1148	18845	12	0	12	0	0	3	3	na	na
1154	17756	0	1	0	0	0	0	na	na	na
1158	20846	0	9	0	0	0	0	na	na	na
1163	1244	0	43	15	0	0	0	na	na	na
1166	17009	21	0	21	0	0	0	na	na	na
1166	35342	0	7	21	0	0	0	na	na	na
1166	35368	0	7	21	0	0	0	na	na	na
1170	12435	0	51	0	0	0	0	na	na	na
1176	30687	0	6	0	0	0	0	na	na	na
1184	34524	10	0	0	0	0	0	na	na	na
1199	40684	0	25	44	0	0	19	19	na	na
1199	40720	44	0	44	0	0	19	19	na	na
1199	40742	8	0	44	0	0	19	19	na	na
1204	8625	0	15	0	0	0	0	na	na	na
1216	6366	18	0	0	0	0	0	na	na	na
1217	37438	44	0	44	0	0	19	19	na	na
1217	37442	44	0	44	0	0	19	19	na	na
1217	37456	44	0	44	0	0	19	19	na	na
1217	37512	8	0	44	0	0	19	19	na	na
1219	28108	0	18	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
1219	28146	1	0	0	0	0	0	na	na	na
1219	28160	0	18	0	0	0	0	na	na	na
1226	8187	0	46	0	0	0	0	na	na	na
1227	23131	0	39	0	0	0	0	na	na	na
1235	28724	0	15	25	0	0	20	20	na	na
1235	28767	0	36	25	25	16.31	20	20	S613M17	29.7cM
1235	28768	0	36	25	25	16.31	20	20	S613M17	29.7cM
1236	7838	4	0	22	0	0	0	na	na	na
1236	7857	0	29	22	22	6.7	0	na	na	na
1236	7864	22	0	22	0	0	0	na	na	na
1236	7880	22	0	22	0	0	0	na	na	na
1236	7896	22	0	22	0	0	0	na	na	na
1239	18138	0	20	0	0	0	0	na	na	na
1241	7487	5	0	0	0	0	0	na	na	na
1244	24493	53	0	53	0	0	0	13	na	na
1246	26227	9	0	0	0	0	0	na	na	na
1247	17421	0	22	0	0	0	0	na	na	na
1247	17460	0	22	0	0	0	0	na	na	na
1250	17416	6	0	0	0	0	0	na	na	na
1250	17461	6	0	0	0	0	0	na	na	na
1271	26849	1	0	1	0	0	10	10	na	na
1271	26888	1	0	1	0	0	10	10	na	na
1271	27034	0	18	0	0	0	0	na	na	na
1271	27058	0	18	0	0	0	0	na	na	na
1272	9096	0	11	0	0	0	0	na	na	na
1277	32865	38	0	38	0	0	0	na	na	na
1284	30981	0	11	0	0	0	0	na	na	na
1288	13310	9	0	0	0	0	0	na	na	na
1288	13357	0	6	0	0	0	0	na	na	na
1290	14151	0	12	0	0	0	0	na	na	na
1291	10890	9	0	0	0	0	0	na	na	na
1294	9719	17	0	17	0	0	0	na	na	na
1294	9751	17	0	17	0	0	0	na	na	na
1296	15055	2	0	0	0	0	0	na	na	na
1296	15060	2	0	0	0	0	0	na	na	na
1298	10414	0	5	0	0	0	0	na	na	na
1303	1948	18	0	18	0	0	0	na	na	na
1303	2003	0	10	18	0	0	0	na	na	na
1303	2017	18	0	18	0	0	0	na	na	na
1304	5547	0	39	26	26	12.37	0	21	T632G11	34.4cM
1304	22737	0	12	26	0	0	15	15	na	na
1313	23446	1	0	1	0	0	0	na	na	na
1313	37395	1	0	1	0	0	0	na	na	na
1313	38749	0	72	1	1	0	0	na	na	na
1313	38757	0	72	1	1	0	0	na	na	na
1313	38809	0	72	1	1	0	0	na	na	na
1315	38373	0	6	0	0	0	0	na	na	na
1319	5207	0	15	10	0	0	0	na	na	na
1319	5221	0	15	10	0	0	0	na	na	na
1319	5224	0	15	10	0	0	0	na	na	na
1319	5225	0	15	10	0	0	0	na	na	na
1319	5227	0	15	10	0	0	0	na	na	na
1319	17324	0	15	10	0	0	0	na	na	na
1319	17334	0	15	10	0	0	0	na	na	na
1336	17428	0	2	0	0	0	0	na	na	na
1336	17470	0	2	0	0	0	0	na	na	na
1341	6389	1	0	1	0	0	0	na	na	na
1341	6416	0	1	1	0	0	0	na	na	na
1341	29489	0	5	0	0	0	0	na	na	na
1344	38373	0	19	23	23.2	41.4	0	na	na	na
1351	31038	0	35	0	0	0	0	na	na	na
1351	32190	11	0	0	0	0	0	na	na	na
1351	32208	0	33	0	0	0	0	na	na	na
1351	32234	11	0	0	0	0	0	na	na	na
1353	21145	0	46	0	0	0	0	na	na	na
1353	21148	0	13	0	0	0	0	na	na	na
1368	13300	0	24	0	0	0	0	na	na	na
1369	15691	0	7	0	0	0	0	na	na	na
1369	15740	0	7	0	0	0	0	na	na	na
1371	1723	4	0	0	0	0	0	na	na	na
1375	153	0	10	0	0	0	0	na	na	na
1378	3565	0	18	0	0	0	0	na	na	na
1378	3620	0	1	0	0	0	0	na	na	na
1387	7645	0	17	12	12.1	3.45	3	3	T004P07	21.0cM
1387	7660	0	17	12	12.1	3.45	3	3	T004P07	21.0cM
1388	22439	0	63	0	0	0	0	na	na	na
1390	22700	46	0	46	0	0	19	19	na	na
1397	28689	0	26	0	0	0	0	na	na	na
1406	7549	0	6	0	0	0	0	na	na	na
1419	28775	0	47	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
1420	4848	0	13	0	0	0	0	na	na	na
1424	21317	0	17	0	0	0	0	na	na	na
1436	21953	0	37	29	29	7.52	9	9	T047M05	24.6cM
1436	21955	0	37	29	29	7.52	9	9	T047M05	24.6cM
1436	21987	29	0	29	0	0	9	9	na	na
1440	33015	12	0	0	0	0	0	na	na	na
1440	33077	12	0	0	0	0	0	na	na	na
1443	2499	0	38	27	27	1.8	0	na	na	na
1448	16429	0	18	0	0	0	0	na	na	na
1453	18398	14	0	0	0	0	0	na	na	na
1461	26767	1	0	0	0	0	0	na	na	na
1464	31632	17	0	17	0	0	0	na	na	na
1467	16724	0	29	22	22	0	6	6	T053A21	4.5cM
1467	16733	4	0	22	0	0	6	6	na	na
1467	16751	0	29	22	22	0	6	6	T053A21	4.5cM
1467	16754	22	0	22	0	0	6	6	na	na
1476	29726	0	18	0	0	0	0	na	na	na
1483	23273	0	4	0	0	0	0	na	na	na
1483	23280	7	0	0	0	0	0	na	na	na
1483	23300	0	4	0	0	0	0	na	na	na
1483	23311	7	0	0	0	0	0	na	na	na
1488	12749	14	0	0	0	0	0	na	na	na
1491	28841	0	32	0	0	0	0	na	na	na
1504	20361	0	8	28	0	0	8	8	na	na
1504	20386	0	8	28	0	0	8	8	na	na
1504	20419	0	8	28	0	0	8	8	na	na
1504	20427	0	8	28	0	0	8	8	T635N11	28.9cM
1520	34828	0	12	26	0	0	0	na	na	na
1520	34860	0	12	26	0	0	0	na	na	na
1524	29097	5	0	0	0	0	0	na	na	na
1530	26022	0	4	0	0	0	0	na	na	na
1540	1418	0	24	16	16	9.71	0	na	na	na
1540	1489	16	0	0	0	0	0	na	na	na
1555	12686	0	26	0	0	0	0	na	na	na
1565	15168	0	44	47	47	0	0	na	na	na
1567	28801	23	0	0	0	0	0	na	na	na
1567	28880	23	0	0	0	0	0	na	na	na
1569	22007	43	0	23	0	0	11	11	na	na
1569	22010	0	19	23	23.2	32.59	11	11	S085G18	26.9cM
1593	31180	33	0	33	0	0	0	na	na	na
1593	31192	0	33	33	33	16.39	0	na	na	na
1593	31201	11	0	33	0	0	0	na	na	na
1594	32036	0	22	0	0	0	0	na	na	na
1622	1136	0	15	10	0	0	0	na	na	na
1622	1169	0	15	10	0	0	0	na	na	na
1623	11681	0	18	0	0	0	0	na	na	na
1623	11702	1	0	0	0	0	0	na	na	na
1624	33547	33	0	0	0	0	0	na	na	na
1625	10604	0	27	40	0	0	0	14	na	na
1625	13006	40	0	40	0	0	14	14	na	na
1626	10957	0	35	0	0	0	0	na	na	na
1635	28863	0	26	23	23	0	0	na	na	na
1635	28870	0	26	23	23	0	0	na	na	na
1636	27002	0	66	28	28	19.08	8	8	T018D03	4.2cM
1642	33377	0	9	0	0	0	0	na	na	na
1644	32533	19	0	19	0	0	0	na	na	na
1646	8742	0	63	0	0	0	0	na	na	na
1652	6754	0	13	0	0	0	0	na	na	na
1661	1344	1	0	0	0	0	0	na	na	na
1661	1414	1	0	0	0	0	0	na	na	na
1670	33602	0	14	0	0	0	0	na	na	na
1682	591	52	60	52	52	50.58	4	4	T652P15	21.8cM
1685	12719	4	0	0	0	0	0	na	na	na
1688	27896	0	9	0	0	0	0	na	na	na
1688	27916	0	23	0	0	0	0	na	na	na
1688	27917	0	9	0	0	0	0	na	na	na
1688	27922	0	9	0	0	0	0	na	na	na
1688	27928	0	23	0	0	0	0	na	na	na
1688	27930	0	9	0	0	0	0	na	na	na
1688	27932	2	0	0	0	0	0	na	na	na
1688	27963	0	23	0	0	0	0	na	na	na
1698	27354	0	12	0	0	0	0	na	na	na
1717	4548	32	0	32	0	0	0	14	na	na
1723	11675	0	18	0	0	0	0	na	na	na
1728	28100	0	11	0	0	0	0	na	na	na
1728	28135	3	0	0	0	0	0	na	na	na
1732	14135	0	47	0	0	0	0	na	na	na
1751	27511	0	73	0	0	0	0	na	na	na
1751	27514	0	73	0	0	0	0	na	na	na
1754	26815	0	12	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
1765	31377	0	58	32	32	13.81	0	na	na	na
1765	31412	0	48	32	0	0	0	na	na	na
1771	24140	0	33	33	33	8.47	0	na	na	na
1776	21030	9	0	0	0	0	0	na	na	na
1776	21087	0	6	0	0	0	0	na	na	na
1785	2313	9	0	0	0	0	0	na	na	na
1785	14915	0	6	0	0	0	0	na	na	na
1804	12403	0	14	0	0	0	0	na	na	na
1804	12450	0	47	0	0	0	0	na	na	na
1804	12460	0	14	0	0	0	0	na	na	na
1804	12475	0	47	0	0	0	0	na	na	na
1816	980	0	23	41	41	8.39	0	na	na	na
1816	1015	41	0	41	0	0	0	na	na	na
1824	17067	0	16	12	12.2	13.69	3	3	T079J21	21.0cM
1824	19822	0	16	12	12.2	13.69	3	3	T079J21	21.0cM
1824	19839	0	17	12	12.1	0	3	3	T079J21	21.0cM
1829	2782	0	65	21	21	2.38	0	16	T062J24	21.7cM
1846	280	11	0	0	0	0	0	na	na	na
1846	28937	6	0	0	0	0	0	na	na	na
1846	28968	0	21	0	0	0	0	na	na	na
1848	18634	0	44	47	47	6.83	0	na	na	na
1850	22596	0	2	0	0	0	0	na	na	na
1850	22597	0	2	0	0	0	0	na	na	na
1852	6669	0	19	23	23.2	0	0	11	T042G04	19.7cM
1853	22723	20	0	0	0	0	0	na	na	na
1868	21499	0	34	8	8	16.47	0	na	na	na
1869	26519	0	67	52	52.4	11.96	0	11	T604I15	0.5cM
1870	6459	0	12	26	0	0	0	na	na	na
1870	6476	0	12	26	0	0	0	na	na	na
1873	10767	2	0	2	0	0	0	na	na	na
1873	10785	0	53	2	0	0	0	na	na	na
1873	10792	0	53	2	0	0	0	na	na	na
1873	10808	8	0	2	0	0	0	na	na	na
1873	10814	0	53	2	0	0	0	na	na	na
1873	10817	0	53	2	0	0	0	na	na	na
1873	10864	2	0	2	0	0	0	na	na	na
1873	10876	2	0	2	0	0	0	na	na	na
1873	10891	2	0	2	0	0	0	na	na	na
1876	24448	0	38	27	27	1.8	0	na	na	na
1879	11698	3	0	0	0	0	0	na	na	na
1884	14133	0	17	0	0	0	0	na	na	na
1885	25388	0	17	12	12.1	0	0	na	na	na
1885	25425	0	16	12	12.2	0	0	na	na	na
1894	7696	2	0	2	0	0	12	12	na	na
1894	7702	2	0	2	0	0	12	12	na	na
1894	7716	0	23	2	0	0	12	12	na	na
1894	7736	2	0	2	0	0	12	12	na	na
1894	10263	2	0	2	0	0	12	12	na	na
1894	10277	2	0	2	0	0	12	12	na	na
1894	23354	2	0	2	0	0	12	12	na	na
1894	23359	2	0	2	0	0	12	12	na	na
1922	13360	0	13	0	0	0	0	na	na	na
1928	24269	0	20	0	0	0	0	na	na	na
1928	24286	0	20	0	0	0	0	na	na	na
1931	28689	0	18	0	0	0	0	na	na	na
1931	28755	0	1	0	0	0	0	na	na	na
1931	28759	0	1	0	0	0	0	na	na	na
1936	17876	35	0	0	0	0	0	na	na	na
1937	11886	0	23	0	0	0	0	na	na	na
1937	11895	0	23	0	0	0	0	na	na	na
1940	23600	9	0	0	0	0	0	na	na	na
1975	22717	9	0	35	0	0	0	na	na	na
1975	22751	35	0	35	0	0	0	na	na	na
1981	12078	24	0	24	0	0	28	28	na	na
1994	21080	0	48	32	0	0	0	na	na	na
1997	29831	23	0	0	0	0	0	na	na	na
2005	26251	23	0	0	0	0	0	na	na	na
2006	1194	30	0	30	0	0	0	na	na	na
2006	1197	30	0	30	0	0	0	na	na	na
2006	1231	14	0	30	0	0	0	na	na	na
2006	1247	30	0	30	0	0	0	na	na	na
2010	26846	0	1	0	0	0	0	na	na	na
2010	26868	1	0	0	0	0	0	na	na	na
2012	3496	0	33	33	33	16.39	0	na	na	na
2012	3525	0	33	33	33	16.39	0	na	na	na
2026	16510	0	12	26	0	0	0	na	na	na
2026	16550	0	39	26	26	0	0	na	na	na
2037	24472	0	67	52	52.4	0	8	8	T610P11	53.1cM
2037	24535	0	67	52	52.4	0	8	8	T610P11	53.1cM
2047	16008	8	0	44	0	0	0	19	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
2047	19681	44	0	44	0	0	0	19	na	na
2047	19690	8	0	44	0	0	0	19	na	na
2047	19702	8	0	44	0	0	0	19	na	na
2047	19704	44	0	44	0	0	0	19	na	na
2047	19709	8	0	44	0	0	0	19	na	na
2050	7755	0	27	6	0	0	0	na	na	na
2050	7762	6	0	6	0	0	0	na	na	na
2050	7783	0	27	6	0	0	0	na	na	na
2050	7785	6	0	6	0	0	0	na	na	na
2056	272	0	21	40	40	0	0	na	na	na
2056	288	40	0	40	0	0	0	na	na	na
2056	315	6	0	40	0	0	0	na	na	na
2057	13491	0	23	0	0	0	0	na	na	na
2063	10940	0	6	35	35	5.27	17	17	S091L04	40.1cM
2063	10952	35	0	35	0	0	17	17	na	na
2063	10955	9	0	35	0	0	17	17	na	na
2066	4785	22	0	22	0	0	6	6	na	na
2066	5229	0	29	22	22	0	6	6	T053A21	4.5cM
2066	5235	22	0	22	0	0	6	6	na	na
2068	10608	5	0	0	0	0	0	na	na	na
2068	10613	0	2	0	0	0	0	na	na	na
2073	3241	0	15	10	0	0	0	20	na	na
2073	11066	10	0	10	0	0	20	20	na	na
2080	13406	0	20	0	0	0	0	na	na	na
2080	29632	0	29	0	0	0	0	na	na	na
2080	29675	0	20	0	0	0	0	na	na	na
2085	9505	0	27	0	0	0	0	na	na	na
2086	21081	29	0	29	0	0	9	9	na	na
2093	9962	40	0	40	0	0	14	14	na	na
2093	27126	40	0	40	0	0	14	14	na	na
2097	2028	0	45	42	42	11.82	22	22	T071P22	39.1cM
2098	3584	18	0	18	0	0	0	na	na	na
2098	3590	18	0	18	0	0	0	na	na	na
2098	3595	0	4	18	0	0	0	na	na	na
2098	3609	18	0	18	0	0	0	na	na	na
2116	11461	0	15	0	0	0	0	na	na	na
2116	11470	25	0	0	0	0	0	na	na	na
2139	29006	26	0	26	0	0	0	na	na	na
2139	29028	26	0	26	0	0	0	na	na	na
2154	29005	41	0	41	0	0	0	na	na	na
2166	26600	17	0	17	0	0	0	22	na	na
2202	8041	28	0	28	0	0	8	8	na	na
2202	9931	0	8	28	0	0	8	8	na	na
2202	9973	0	66	28	28	16.87	0	8	T018D03	4.2cM
2202	16458	0	8	28	0	0	0	8	na	na
2227	15844	0	7	0	0	0	0	na	na	na
2240	6481	11	0	0	0	0	0	na	na	na
2240	6508	11	0	0	0	0	0	na	na	na
2240	6529	0	35	0	0	0	0	na	na	na
2240	6541	33	0	0	0	0	0	na	na	na
2250	23033	18	0	0	0	0	0	na	na	na
2251	27932	0	15	0	0	0	0	na	na	na
2257	1941	0	15	10	0	0	0	na	na	na
2257	1944	0	15	10	0	0	0	na	na	na
2280	7466	0	32	49	0	0	0	11	na	na
2280	7488	0	68	49	49	5.89	0	11	S079O18	61.9cM
2290	27609	1	0	1	0	0	1	1	na	na
2290	27618	1	0	1	0	0	1	1	na	na
2290	27625	1	0	1	0	0	1	1	na	na
2290	27703	1	0	1	0	0	1	1	na	na
2313	23669	6	0	40	0	0	0	na	na	na
2313	23695	6	0	40	0	0	0	na	na	na
2313	23718	40	0	40	0	0	0	na	na	na
2318	19900	4	0	0	0	0	0	na	na	na
2339	27511	0	43	15	0	0	0	na	na	na
2342	21124	29	0	29	0	0	0	na	na	na
2356	17709	0	41	0	0	0	0	na	na	na
2360	27448	31	0	31	0	0	0	5	na	na
2360	27457	31	0	31	0	0	0	5	na	na
2360	27526	0	42	31	0	0	0	5	na	na
2360	27562	31	0	31	0	0	0	5	na	na
2363	9599	2	0	0	0	0	0	na	na	na
2381	10993	7	0	0	0	0	0	na	na	na
2381	11060	0	4	0	0	0	0	na	na	na
2384	1247	12	0	0	0	0	0	na	na	na
2385	422	0	49	13	13	33.72	0	18	T075O24	2.6cM
2385	428	0	49	13	13	33.72	0	na	na	na
2385	473	0	49	13	13	33.72	18	na	na	na
2397	5276	28	0	28	0	0	0	na	na	na
2421	2204	19	0	19	0	0	0	10	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
2421	25775	0	17	19	0	0	3	3	na	na
2422	12494	7	0	0	0	0	0	na	na	na
2422	12496	7	0	0	0	0	0	na	na	na
2423	18192	5	0	0	0	0	0	na	na	na
2459	9794	0	1	0	0	0	0	na	na	na
2459	9834	1	0	1	0	0	0	na	na	na
2459	9835	1	0	0	0	0	0	na	na	na
2461	19992	0	25	0	0	0	0	na	na	na
2461	20048	0	25	0	0	0	0	na	na	na
2491	8570	12	0	0	0	0	0	na	na	na
2493	26481	0	19	23	23.2	36.95	0	na	na	na
2493	26519	23	0	23	0	0	0	na	na	na
2507	20529	0	1	0	0	0	0	na	na	na
2507	20568	0	1	0	0	0	0	na	na	na
2514	11329	0	38	27	27	1.8	0	na	na	na
2514	11349	0	38	27	27	1.8	0	na	na	na
2515	24473	16	0	0	0	0	0	na	na	na
2520	6131	1	0	0	0	0	0	na	na	na
2523	11719	14	0	0	0	0	0	na	na	na
2524	8355	13	0	0	0	0	0	na	na	na
2530	18307	0	33	33	33	7.39	0	na	na	na
2545	25796	0	14	0	0	0	0	na	na	na
2568	1006	0	20	0	0	0	0	na	na	na
2568	1048	0	29	0	0	0	0	na	na	na
2580	18624	0	38	27	27	1.8	0	na	na	na
2580	18629	0	38	27	27	1.8	0	na	na	na
2585	8694	0	12	0	0	0	0	na	na	na
2593	10699	0	42	37	0	0	26	26	na	na
2593	17519	37	0	37	0	0	0	na	na	na
2594	16367	0	1	0	0	0	0	na	na	na
2594	16497	0	1	0	0	0	0	na	na	na
2596	2873	7	0	0	0	0	0	na	na	na
2611	19218	0	28	0	0	0	0	na	na	na
2615	10205	0	28	42	0	0	0	7	na	na
2615	10223	42	0	42	0	0	0	7	na	na
2632	16310	36	0	36	0	0	14	14	na	na
2632	16446	6	0	36	0	0	14	14	na	na
2648	487	52	60	52	52.3	12.28	5	5	T035M24	14.8cM
2657	24072	19	0	19	0	0	0	na	na	na
2657	24117	19	0	19	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
2659	11120	0	38	27	27	1.8	0	na	na	na
2677	19944	0	9	0	0	0	0	na	na	na
2686	18856	12	0	0	0	0	0	na	na	na
2698	24600	0	44	47	47	6.83	25	25	T640C15	0.0cM
2698	24628	0	44	47	47	6.83	25	25	T640C15	0.0cM
2698	24637	0	44	47	47	6.83	25	25	T640C15	0.0cM
2698	24988	0	11	47	0	0	25	25	na	na
2703	17049	0	27	0	0	0	0	na	na	na
2703	17075	0	21	0	0	0	0	na	na	na
2704	24132	10	0	0	0	0	0	na	na	na
2704	24161	25	0	0	0	0	0	na	na	na
2704	24163	10	0	0	0	0	0	na	na	na
2718	5752	29	0	29	0	0	0	na	na	na
2725	14021	0	27	0	0	0	0	na	na	na
2725	14072	0	21	0	0	0	0	na	na	na
2725	14105	6	0	0	0	0	0	na	na	na
2728	16786	5	0	0	0	0	0	na	na	na
2746	17783	0	20	4	0	0	6	6	na	na
2746	20639	0	22	0	0	0	0	na	na	na
2747	405	37	0	37	0	0	0	26	na	na
2747	422	0	52	37	37	13.99	26	na	na	na
2755	4857	19	0	19	0	0	0	na	na	na
2783	8255	15	0	15	0	0	0	na	na	na
2783	8299	15	0	15	0	0	0	na	na	na
2794	7008	0	25	0	0	0	0	na	na	na
2797	18134	0	28	17	0	0	0	na	na	na
2797	18172	0	28	17	0	0	0	na	na	na
2820	18235	0	8	28	0	0	8	8	na	na
2820	18317	0	8	28	0	0	8	8	na	na
2820	18343	28	0	28	0	0	8	8	na	na
2820	24319	0	8	28	0	0	0	na	na	na
2820	24336	0	8	28	0	0	0	na	na	na
2824	21011	0	43	15	0	0	0	na	na	na
2824	21014	0	43	15	0	0	0	na	na	na
2824	21016	0	43	15	0	0	0	na	na	na
2824	21017	0	43	15	0	0	0	na	na	na
2843	1134	45	0	45	0	0	0	na	na	na
2859	10541	34	0	34	0	0	4	4	na	na
2873	347	0	28	17	0	0	0	22	na	na
2873	380	0	28	17	0	0	22	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
2885	1595	0	22	0	0	0	0	na	na	na
2896	838	0	17	0	0	0	0	na	na	na
2897	23963	0	28	17	0	0	0	15	na	na
2897	24004	0	28	17	0	0	0	9	na	na
2897	24023	0	28	17	0	0	0	27	na	na
2916	8585	0	24	16	16	9.71	0	25	T636E18	29.3cM
2916	8606	0	24	16	16	9.71	0	25	T636E18	29.3cM
2916	8617	16	0	16	0	0	0	25	na	na
2916	8635	3	0	0	0	0	0	na	na	na
2916	21042	0	11	0	0	0	0	na	na	na
2944	10554	0	27	0	0	0	0	na	na	na
2949	7683	47	0	47	0	0	25	25	na	na
2955	18897	0	2	0	0	0	0	na	na	na
2959	13164	0	19	23	23.2	32.59	0	na	na	na
2959	13228	0	19	23	23.2	32.59	0	na	na	na
2960	12869	9	0	0	0	0	0	na	na	na
2968	17332	0	31	0	0	0	0	na	na	na
2976	4609	5	0	0	0	0	0	na	na	na
3034	16579	0	36	25	25	16.31	0	4	T001F04	0.0cM
3034	16594	0	36	25	25	16.31	0	4	T001F04	0.0cM
3061	15229	4	0	0	0	0	0	na	na	na
3067	3502	0	49	13	13	25.26	18	18	T096I03	19.0cM
3069	22141	0	30	0	0	0	0	na	na	na
3076	14079	0	20	0	0	0	0	na	na	na
3079	17050	9	0	0	0	0	0	na	na	na
3079	17055	0	6	0	0	0	0	na	na	na
3088	7528	0	45	42	42	16.27	0	22	S094M17	54.8cM
3110	17122	13	0	0	0	0	0	na	na	na
3118	15787	0	1	0	0	0	0	na	na	na
3120	4743	0	11	0	0	0	0	na	na	na
3120	16516	3	0	0	0	0	0	na	na	na
3120	16517	3	0	0	0	0	0	na	na	na
3129	5014	0	30	0	0	0	0	na	na	na
3150	4124	0	12	26	0	0	0	na	na	na
3150	4136	0	39	26	26	0	0	na	na	na
3150	4158	0	12	26	0	0	0	na	na	na
3150	4175	0	39	26	26	0	0	na	na	na
3156	14262	0	35	0	0	0	0	na	na	na
3156	14269	11	0	0	0	0	0	na	na	na
3158	12589	0	4	0	0	0	0	na	na	na
3168	2041	0	5	0	0	0	0	na	na	na
3169	10472	27	0	28	0	0	0	na	na	na
3169	10478	28	0	28	0	0	0	na	na	na
3169	10503	28	0	28	0	0	0	na	na	na
3199	5659	0	8	28	0	0	8	8	na	na
3204	21245	0	40	0	0	0	0	na	na	na
3206	575	0	2	0	0	0	0	na	na	na
3206	631	0	2	0	0	0	0	na	na	na
3207	13297	9	0	0	0	0	0	na	na	na
3207	13302	9	0	0	0	0	0	na	na	na
3207	13318	0	6	0	0	0	0	na	na	na
3207	13325	9	0	0	0	0	0	na	na	na
3213	15315	18	0	0	0	0	0	na	na	na
3222	21546	0	8	28	0	0	8	8	na	na
3222	21554	0	8	28	0	0	8	8	na	na
3240	4869	0	5	0	0	0	0	na	na	na
3240	5760	0	5	0	0	0	0	na	na	na
3240	18398	0	5	0	0	0	0	na	na	na
3240	18428	0	2	0	0	0	0	na	na	na
3312	401	0	16	12	12.2	0	0	na	na	na
3312	428	0	16	12	12.2	0	0	na	na	na
3312	447	12	0	12	0	0	0	na	na	na
3314	20990	0	26	0	0	0	0	na	na	na
3333	971	10	0	0	0	0	0	na	na	na
3333	21238	10	0	0	0	0	0	na	na	na
3349	7803	0	27	0	0	0	0	na	na	na
3349	7817	0	27	0	0	0	0	na	na	na
3353	8583	0	47	0	0	0	0	na	na	na
3355	453	1	0	1	0	0	0	na	na	na
3355	459	1	0	1	0	0	0	na	na	na
3355	501	0	1	1	0	0	0	na	na	na
3355	506	0	1	1	0	0	0	na	na	na
3355	537	1	0	1	0	0	0	na	na	na
3360	3821	0	1	0	0	0	0	na	na	na
3370	19957	12	0	0	0	0	0	na	na	na
3386	14811	0	51	0	0	0	0	na	na	na
3401	6281	0	16	0	0	0	0	na	na	na
3405	2939	24	0	24	0	0	28	28	na	na
3421	6617	0	24	0	0	0	0	na	na	na
3421	6691	3	0	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
3429	8989	0	4	0	0	0	0	na	na	na
3429	11963	0	4	0	0	0	0	na	na	na
3473	10409	0	2	0	0	0	0	na	na	na
3473	10419	0	2	0	0	0	0	na	na	na
3473	11045	5	0	0	0	0	0	na	na	na
3499	12082	0	28	0	0	0	0	na	na	na
3510	19348	0	14	0	0	0	0	na	na	na
3510	19368	0	14	0	0	0	0	na	na	na
3511	7716	0	70	52	52.2	8.35	0	16	T022I06	6.9cM
3525	5473	0	13	0	0	0	0	na	na	na
3531	13146	0	43	15	0	0	0	6	na	na
3541	8491	2	0	0	0	0	0	na	na	na
3545	20199	10	0	0	0	0	0	na	na	na
3546	18369	27	0	27	0	0	0	na	na	na
3546	18374	27	0	27	0	0	0	na	na	na
3546	18418	27	0	27	0	0	0	na	na	na
3546	18422	27	0	27	0	0	0	na	na	na
3547	6947	0	29	0	0	0	0	na	na	na
3547	6967	52	60	52	52.3	0	0	na	na	na
3548	15992	0	10	0	0	0	0	na	na	na
3548	16006	0	10	0	0	0	0	na	na	na
3548	16668	0	10	0	0	0	0	na	na	na
3548	16669	0	10	0	0	0	0	na	na	na
3552	7304	0	65	21	21	0	0	na	na	na
3552	7330	0	65	21	21	0	0	na	na	na
3555	11239	45	0	45	0	0	0	27	na	na
3567	9958	0	11	3	0	0	0	na	na	na
3567	9977	0	11	3	0	0	0	na	na	na
3567	10015	16	0	3	0	0	0	na	na	na
3567	10036	0	11	3	0	0	0	na	na	na
3568	17959	0	14	0	0	0	0	na	na	na
3573	14039	0	16	12	12.2	13.69	3	3	S091B11	8.4cM
3573	14048	12	0	12	0	0	3	3	na	na
3574	6700	0	33	33	33	16.39	7	7	T053B14	22.4cM
3587	15681	0	74	49	0	0	0	23	na	na
3587	15708	0	68	49	49	0	0	23	T630K11	40.4cM
3597	11222	3	0	0	0	0	0	na	na	na
3597	11227	3	0	0	0	0	0	na	na	na
3612	9416	5	0	0	0	0	0	na	na	na
3614	2197	10	0	25	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
3614	2202	0	36	25	25	11.96	0	na	na	na
3638	5766	0	4	0	0	0	0	na	na	na
3658	2705	0	3	0	0	0	0	na	na	na
3663	1797	0	2	0	0	0	0	na	na	na
3663	8001	0	5	0	0	0	0	na	na	na
3663	13436	0	2	0	0	0	0	na	na	na
3674	7402	21	0	21	0	0	20	20	na	na
3674	7426	21	0	21	0	0	20	20	na	na
3674	7463	21	0	21	0	0	20	20	na	na
3674	7464	21	0	21	0	0	20	20	na	na
3689	784	0	30	0	0	0	0	na	na	na
3703	5945	40	0	40	0	0	0	na	na	na
3703	6007	0	21	40	40	4.55	0	na	na	na
3716	11732	10	0	0	0	0	0	na	na	na
3727	4622	0	3	0	0	0	0	na	na	na
3793	7785	1	0	0	0	0	0	na	na	na
3800	5941	0	50	11	11	9.33	0	na	na	na
3800	5960	0	50	11	11	9.33	0	na	na	na
3800	13501	0	50	11	11	9.33	7	7	T006J12	43.0cM
3800	13586	0	50	11	11	11.5	7	7	T006J12	43.0cM
3801	9052	7	0	0	0	0	0	na	na	na
3801	9064	7	0	0	0	0	0	na	na	na
3801	17281	0	4	0	0	0	0	na	na	na
3807	1027	0	8	28	0	0	0	8	na	na
3808	16129	0	7	0	0	0	0	na	na	na
3886	11088	1	0	0	0	0	0	na	na	na
3923	12395	0	5	0	0	0	0	na	na	na
3923	12420	5	0	0	0	0	0	na	na	na
3934	8007	21	0	21	0	0	28	28	na	na
3934	8008	21	0	21	0	0	28	28	na	na
3942	10514	3	0	0	0	0	0	na	na	na
3942	10521	0	11	0	0	0	0	na	na	na
3945	1862	37	0	37	0	0	0	na	na	na
3948	13881	0	6	0	0	0	0	na	na	na
3948	13937	0	6	0	0	0	0	na	na	na
3949	17156	0	2	0	0	0	0	na	na	na
3955	5877	32	0	32	0	0	24	24	na	na
3955	5878	32	0	32	0	0	24	24	na	na
3955	5883	32	0	32	0	0	24	24	na	na
3955	6050	0	58	32	32	16.02	24	24	T029D18	29.0cM

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
3957	5814	0	1	0	0	0	0	na	na	na
3959	17371	22	0	22	0	0	6	6	na	na
3968	12525	0	37	29	29	5.35	0	9	T030J03	22.5cM
3969	14527	7	0	7	0	0	0	15	na	na
3969	14647	0	10	7	7	0	0	15	T058J12	1.6cM
3996	17816	0	4	0	0	0	0	na	na	na
4022	1524	0	13	0	0	0	0	na	na	na
4022	12816	0	11	0	0	0	0	na	na	na
4061	11649	41	0	41	0	0	0	na	na	na
4068	17579	0	21	0	0	0	0	na	na	na
4074	1276	0	5	0	0	0	0	na	na	na
4077	13430	0	23	0	0	0	0	na	na	na
4085	17890	0	56	50	50.2	0	0	na	na	na
4113	11624	4	0	0	0	0	0	na	na	na
4113	17670	4	0	0	0	0	0	na	na	na
4113	17704	0	29	0	0	0	0	na	na	na
4113	17709	22	0	0	0	0	0	na	na	na
4113	17732	4	0	0	0	0	0	na	na	na
4113	17746	4	0	0	0	0	0	na	na	na
4121	17560	0	3	14	0	0	5	5	na	na
4121	17593	14	0	14	0	0	5	5	na	na
4129	13922	44	0	44	0	0	19	19	na	na
4132	6168	0	66	28	28	23.53	8	8	T058G05	0.0cM
4132	6204	0	8	28	0	0	8	8	na	na
4132	6213	0	66	28	28	23.53	8	8	T058G05	0.0cM
4154	3647	19	0	19	0	0	10	10	na	na
4156	13020	0	36	25	25	0	20	20	B014E18	53.1cM
4156	13030	0	36	25	25	0	20	20	B014E18	53.1cM
4156	13035	0	36	25	25	0	20	20	B014E18	53.1cM
4156	13070	10	0	25	0	0	20	20	na	na
4175	782	2	0	0	0	0	0	na	na	na
4185	13520	0	18	0	0	0	0	na	na	na
4189	12833	6	0	0	0	0	0	na	na	na
4189	12855	6	0	0	0	0	0	na	na	na
4189	12860	6	0	0	0	0	0	na	na	na
4191	17544	0	37	29	29	7.52	0	10	T036P11	31.6cM
4194	15791	0	13	0	0	0	0	na	na	na
4204	6165	0	52	0	0	0	0	na	na	na
4206	4309	0	10	0	0	0	0	na	na	na
4215	7302	0	37	29	29	3.13	0	na	na	na
4215	7306	29	0	29	0	0	0	na	na	na
4222	10931	53	0	53	0	0	0	28	na	na
4222	10964	0	54	53	0	0	0	28	na	na
4222	10978	0	54	53	0	0	0	28	na	na
4222	10987	0	54	53	0	0	0	28	na	na
4231	6026	0	45	42	42	16.27	22	22	B513O16	28.0cM
4234	16100	0	10	0	0	0	0	na	na	na
4260	8774	8	0	0	0	0	0	na	na	na
4260	8835	0	25	0	0	0	0	na	na	na
4269	7064	23	0	0	0	0	0	na	na	na
4281	1241	7	0	0	0	0	0	na	na	na
4293	11387	24	0	24	0	0	0	na	na	na
4293	11389	0	64	24	24	29.96	0	na	na	na
4329	7807	0	63	15	15	7.92	0	14	T070O22	5.3cM
4329	7892	0	43	15	0	0	0	13	na	na
4329	7907	0	43	15	0	0	0	13	na	na
4333	11992	0	2	0	0	0	0	na	na	na
4333	11997	0	2	0	0	0	0	na	na	na
4346	4469	0	17	12	12.1	0	3	3	T028M20	25.2cM
4346	4482	0	16	12	12.2	9.33	3	3	T028M20	25.2cM
4354	627	17	0	17	0	0	0	na	na	na
4370	12109	46	0	46	0	0	0	19	na	na
4382	10395	6	0	0	0	0	0	na	na	na
4383	11071	0	24	0	0	0	0	na	na	na
4409	6097	0	39	0	0	0	0	na	na	na
4445	16664	0	2	0	0	0	0	na	na	na
4445	16716	0	2	0	0	0	0	na	na	na
4454	11543	25	0	25	0	0	0	na	na	na
4465	14982	16	0	47	0	0	0	25	na	na
4465	14993	47	0	47	0	0	0	25	na	na
4504	8795	0	65	21	21	0	0	na	na	na
4504	8807	0	65	21	21	0	0	na	na	na
4506	8720	0	14	0	0	0	0	na	na	na
4512	4214	5	0	0	0	0	0	na	na	na
4522	15303	0	11	3	0	0	0	na	na	na
4522	15307	0	11	3	0	0	0	na	na	na
4522	15326	16	0	3	0	0	0	na	na	na
4522	15329	0	11	3	0	0	0	na	na	na
4523	8086	0	10	0	0	0	0	na	na	na
4536	570	0	67	52	52.4	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
4537	11903	0	32	53	0	0	0	na	na	na
4537	11907	53	0	53	0	0	0	na	na	na
4537	11908	0	32	53	0	0	0	na	na	na
4537	11948	0	32	53	0	0	0	na	na	na
4559	9951	0	7	0	0	0	0	na	na	na
4559	9983	0	3	0	0	0	0	na	na	na
4572	10891	0	35	33	0	0	0	7	na	na
4572	12054	11	0	33	0	0	7	7	na	na
4572	12090	0	33	33	33	16.39	7	7	B001F15	21.4cM
4572	12117	11	0	33	0	0	7	7	na	na
4572	12125	33	0	33	0	0	7	7	na	na
4573	5779	0	31	0	0	0	0	na	na	na
4576	13326	0	5	0	0	0	0	na	na	na
4576	16728	5	0	0	0	0	0	na	na	na
4581	1669	35	0	0	0	0	0	na	na	na
4641	792	45	0	45	0	0	0	na	na	na
4641	812	45	0	45	0	0	0	na	na	na
4643	2464	0	6	0	0	0	0	na	na	na
4659	2116	0	37	29	29	7.52	0	na	na	na
4659	13053	0	37	29	29	7.52	9	9	T613P10	34.5cM
4684	11343	48	0	48	0	0	13	13	na	na
4684	11358	48	0	48	0	0	13	13	na	na
4684	11391	0	4	48	0	0	13	13	na	na
4684	11394	7	0	48	0	0	13	13	na	na
4684	11403	7	0	48	0	0	13	13	na	na
4715	2461	0	50	11	11	0	0	na	na	na
4715	2468	0	50	11	11	0	0	na	na	na
4715	2504	0	50	11	11	0	0	na	na	na
4715	2536	0	50	11	11	0	0	na	na	na
4715	10768	11	0	11	0	0	0	na	na	na
4725	10797	12	0	0	0	0	0	na	na	na
4725	10799	12	0	0	0	0	0	na	na	na
4732	13653	0	30	0	0	0	0	na	na	na
4733	11160	0	41	0	0	0	0	na	na	na
4739	6401	1	0	0	0	0	0	na	na	na
4744	15068	35	0	0	0	0	0	na	na	na
4745	15830	0	24	16	16	9.71	25	25	T066K06	38.8cM
4745	15871	0	24	16	16	9.71	25	25	T066K06	38.8cM
4745	15876	0	24	16	16	9.71	25	25	T066K06	38.8cM
4759	659	18	0	18	0	0	0	na	na	na
4759	691	18	0	18	0	0	0	na	na	na
4783	8651	4	0	0	0	0	0	na	na	na
4803	7718	0	1	0	0	0	0	na	na	na
4803	7731	1	0	0	0	0	0	na	na	na
4816	12370	11	0	0	0	0	0	na	na	na
4858	2974	25	0	25	0	0	18	18	na	na
4858	2980	0	36	25	25	25.64	18	18	T638K08	45.5cM
4868	15496	0	48	32	0	0	0	na	na	na
4873	562	0	5	0	0	0	0	na	na	na
4889	9133	0	29	0	0	0	0	na	na	na
4889	10152	4	0	0	0	0	0	na	na	na
4889	10171	4	0	0	0	0	0	na	na	na
4909	8285	1	0	0	0	0	0	na	na	na
4909	8286	1	0	0	0	0	0	na	na	na
4911	13754	0	35	0	0	0	0	na	na	na
4925	9502	0	45	42	42	16.27	0	na	na	na
4930	5724	27	0	27	0	0	15	15	na	na
4930	5750	27	0	27	0	0	15	15	na	na
4931	2518	3	0	0	0	0	0	na	na	na
4931	2519	3	0	0	0	0	0	na	na	na
4933	462	20	0	0	0	0	0	na	na	na
4934	2829	0	38	27	27	1.8	0	na	na	na
4940	13819	1	0	0	0	0	0	na	na	na
4947	13887	4	0	0	0	0	0	na	na	na
4949	2682	52	60	52	0	0	0	na	na	na
4949	2711	0	9	0	0	0	0	na	na	na
4949	5168	2	0	0	0	0	0	na	na	na
4949	5236	0	23	0	0	0	0	na	na	na
4950	13782	0	33	33	33	2.94	7	7	T639A10	3.7cM
4950	13865	33	0	33	0	0	7	7	na	na
4958	14788	0	22	0	0	0	0	na	na	na
4961	574	7	0	7	0	0	13	13	na	na
4961	616	0	10	7	7	9.17	13	13	T058B18	20.3cM
4977	1444	0	28	0	0	0	0	na	na	na
4990	3623	0	34	0	0	0	0	na	na	na
4990	13835	8	0	0	0	0	0	na	na	na
4990	13868	46	0	0	0	0	0	na	na	na
4990	13903	0	25	0	0	0	0	na	na	na
4992	9913	0	10	0	0	0	0	na	na	na
5002	10328	0	12	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
5023	4933	0	34	8	8	14.3	19	19	T631D24	17.1cM
5023	4971	0	34	8	8	14.3	19	19	T631D24	17.1cM
5044	6666	0	2	34	0	0	0	na	na	na
5044	6677	5	0	34	0	0	0	na	na	na
5044	15253	34	0	34	0	0	0	na	na	na
5044	15255	0	2	34	0	0	0	na	na	na
5044	15269	0	5	34	34	2.63	0	na	na	na
5044	15279	5	0	34	0	0	0	na	na	na
5044	15315	0	2	34	0	0	0	na	na	na
5044	15361	34	0	34	0	0	0	na	na	na
5112	13909	0	71	0	0	0	0	na	na	na
5138	13543	0	51	0	0	0	0	na	na	na
5156	14032	0	13	0	0	0	0	na	na	na
5166	10280	6	0	0	0	0	0	na	na	na
5168	14636	0	1	0	0	0	0	na	na	na
5169	13250	43	0	0	0	0	0	na	na	na
5200	397	0	21	0	0	0	0	na	na	na
5200	475	6	0	0	0	0	0	na	na	na
5216	1701	0	56	50	50.2	6.26	0	na	na	na
5216	1754	0	56	50	50.2	6.26	0	na	na	na
5227	4204	0	5	5	5	4.45	0	4	T624I13	36.2cM
5227	4252	0	5	5	5	4.45	0	4	T624I13	36.2cM
5227	4261	0	5	5	5	4.45	0	4	T624I13	36.2cM
5231	8345	12	0	12	0	0	0	24	na	na
5231	8352	0	16	12	12.2	13.69	0	24	T602I07	51.6cM
5231	8373	0	16	12	12.2	13.69	0	24	T602I07	51.6cM
5231	8395	0	16	12	12.2	13.69	0	24	T602I07	51.6cM
5234	13895	37	0	37	0	0	0	14	na	na
5237	884	0	59	50	50	0	0	na	na	na
5237	908	0	59	50	50	0	0	na	na	na
5237	967	0	59	50	50	0	0	na	na	na
5244	12292	0	1	0	0	0	0	na	na	na
5282	2527	0	48	32	0	0	24	24	na	na
5282	8812	32	0	32	0	0	0	na	na	na
5282	8844	0	48	32	0	0	0	na	na	na
5282	8846	32	0	32	0	0	0	na	na	na
5282	8872	32	0	32	0	0	0	na	na	na
5282	8875	32	0	32	0	0	0	na	na	na
5300	13992	17	0	17	0	0	0	na	na	na
5300	14031	17	0	17	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
5388	1716	6	0	0	0	0	0	na	na	na
5388	1765	6	0	0	0	0	0	na	na	na
5388	1777	0	27	0	0	0	0	na	na	na
5435	7207	0	19	23	23.2	32.59	11	11	T611E03	30.6cM
5460	12813	0	16	0	0	0	0	na	na	na
5473	13664	3	0	0	0	0	0	na	na	na
5473	13701	0	11	0	0	0	0	na	na	na
5494	11386	38	0	38	0	0	0	15	na	na
5515	5862	0	44	47	47	6.83	0	na	na	na
5515	5879	0	44	47	47	6.83	0	na	na	na
5515	5902	47	0	47	0	0	0	na	na	na
5520	12071	19	0	19	0	0	10	10	na	na
5520	12107	0	1	19	19	3.45	10	10	T035K04	20.6cM
5526	12499	5	0	0	0	0	0	na	na	na
5546	9171	0	33	33	33	19.52	0	11	T639N23	55.0cM
5546	9203	33	0	33	0	0	0	11	na	na
5551	753	0	13	38	0	0	0	na	na	na
5551	786	0	13	38	0	0	0	na	na	na
5563	4301	3	0	0	0	0	0	na	na	na
5563	4370	3	0	0	0	0	0	na	na	na
5572	1450	0	9	0	0	0	0	na	na	na
5572	1485	0	23	0	0	0	0	na	na	na
5572	1491	0	9	0	0	0	0	na	na	na
5602	14053	0	40	0	0	0	0	na	na	na
5602	14133	0	41	0	0	0	0	na	na	na
5608	11108	2	0	0	0	0	0	na	na	na
5611	6401	0	28	0	0	0	0	na	na	na
5630	3546	2	0	0	0	0	0	na	na	na
5631	8269	51	0	51	0	0	9	9	na	na
5631	8275	51	0	51	0	0	9	9	na	na
5647	7454	0	8	28	0	0	0	28	na	na
5658	6609	5	0	0	0	0	0	na	na	na
5687	13145	0	14	0	0	0	0	na	na	na
5691	13610	7	0	7	0	0	0	na	na	na
5691	13614	18	0	7	0	0	0	na	na	na
5691	13633	0	4	0	0	0	0	na	na	na
5702	7144	0	2	0	0	0	0	na	na	na
5716	14135	0	46	0	0	0	0	na	na	na
5720	6918	53	0	53	0	0	28	28	na	na
5735	6739	0	5	5	5	29.64	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
5735	6754	0	5	5	5	29.64	0	na	na	na
5735	6795	5	0	5	0	0	0	na	na	na
5760	11998	0	22	0	0	0	0	na	na	na
5797	170	0	22	0	0	0	0	na	na	na
5799	1940	22	0	22	0	0	0	na	na	na
5799	1971	4	0	22	0	0	0	na	na	na
5804	1387	0	8	28	0	0	8	8	na	na
5804	8034	28	0	28	0	0	8	8	na	na
5805	8107	0	45	42	42	16.27	0	na	na	na
5839	13531	0	41	0	0	0	0	na	na	na
5843	12787	9	0	0	0	0	0	na	na	na
5857	11515	38	0	38	0	0	18	18	na	na
5862	9066	3	0	0	0	0	0	na	na	na
5862	9094	3	0	0	0	0	0	na	na	na
5908	5549	4	0	0	0	0	0	na	na	na
5909	11904	8	0	0	0	0	0	na	na	na
5912	7874	23	0	0	0	0	0	na	na	na
5921	2775	0	13	0	0	0	0	na	na	na
5942	3265	0	4	0	0	0	0	na	na	na
5958	1922	10	0	0	0	0	0	na	na	na
5958	1998	10	0	0	0	0	0	na	na	na
5966	12890	0	34	8	8	0	0	12	S006F19	0.0cM
5986	12748	25	0	25	0	0	0	na	na	na
5986	12759	25	0	25	0	0	0	na	na	na
5986	12827	10	0	25	0	0	0	na	na	na
6019	4897	0	59	50	50	0	0	na	na	na
6039	6251	0	44	47	47	6.83	0	na	na	na
6039	6257	0	44	47	47	6.83	0	na	na	na
6039	6263	0	11	47	0	0	0	na	na	na
6039	6268	47	0	47	0	0	0	na	na	na
6039	6314	47	0	47	0	0	0	na	na	na
6041	9057	0	13	38	0	0	0	na	na	na
6041	9064	38	0	38	0	0	0	na	na	na
6052	2641	0	14	0	0	0	0	na	na	na
6061	6292	11	0	0	0	0	0	na	na	na
6061	6298	11	0	0	0	0	0	na	na	na
6081	4969	0	26	23	23	0	0	na	na	na
6081	5030	23	0	23	0	0	21	21	na	na
6081	5045	43	0	23	0	0	21	21	na	na
6081	5049	0	19	23	23.2	11.96	21	21	T068N16	32.8cM

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
6081	5050	0	19	23	23.2	11.96	21	21	T068N16	32.8cM
6092	12242	0	1	0	0	0	0	na	na	na
6096	8554	0	11	0	0	0	0	na	na	na
6096	8560	0	11	0	0	0	0	na	na	na
6104	8527	29	0	29	0	0	9	9	na	na
6104	8566	29	0	29	0	0	9	9	na	na
6141	5010	0	33	33	33	2.94	0	na	na	na
6160	1587	11	0	0	0	0	0	na	na	na
6188	5099	43	0	0	0	0	0	na	na	na
6207	5647	0	41	0	0	0	0	na	na	na
6207	5651	0	41	0	0	0	0	na	na	na
6208	7718	0	47	0	0	0	0	na	na	na
6240	3626	23	0	0	0	0	0	na	na	na
6332	2034	0	17	12	12.1	0	0	na	na	na
6332	2088	0	17	12	12.1	0	0	na	na	na
6354	10229	0	75	0	0	0	0	na	na	na
6363	11307	24	0	24	0	0	0	na	na	na
6363	11312	24	0	24	0	0	0	na	na	na
6363	11318	24	0	24	0	0	0	na	na	na
6363	11327	0	32	24	0	0	0	na	na	na
6363	11337	0	32	24	0	0	0	na	na	na
6444	4315	0	2	0	0	0	0	na	na	na
6446	1121	0	12	26	0	0	0	na	na	na
6446	1144	0	12	26	0	0	0	na	na	na
6450	407	10	0	10	0	0	0	22	na	na
6450	458	0	15	10	0	0	1	na	na	na
6454	12270	0	69	0	0	0	0	na	na	na
6479	450	0	72	1	1	3.13	0	na	na	na
6563	6908	5	0	34	0	0	0	na	na	na
6563	6939	34	0	34	0	0	0	na	na	na
6563	6953	0	2	34	0	0	0	na	na	na
6569	6498	0	3	0	0	0	0	na	na	na
6576	537	14	0	14	0	0	0	na	na	na
6576	6378	14	0	14	0	0	0	23	na	na
6576	6436	14	0	14	0	0	0	23	na	na
6603	6627	0	25	0	0	0	0	na	na	na
6605	11286	28	0	28	0	0	0	na	na	na
6605	11292	0	66	28	28	23.53	0	na	na	na
6637	8616	0	14	0	0	0	0	na	na	na
6637	8619	0	14	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
6646	2120	0	50	11	11	0	0	na	na	na
6659	514	23	0	0	0	0	0	na	na	na
6691	10834	20	0	0	0	0	0	na	na	na
6708	3700	10	0	10	0	0	0	na	na	na
6708	3714	0	15	10	0	0	0	na	na	na
6714	7187	0	1	0	0	0	0	na	na	na
6714	7251	0	18	0	0	0	0	na	na	na
6775	502	0	2	0	0	0	0	na	na	na
6797	11994	0	7	0	0	0	0	na	na	na
6798	6025	26	0	26	0	0	21	21	na	na
6802	11797	32	0	32	0	0	13	13	na	na
6807	10348	0	40	0	0	0	0	na	na	na
6808	5995	0	16	0	0	0	0	na	na	na
6827	5703	2	0	0	0	0	0	na	na	na
6855	2138	0	41	44	0	0	0	na	na	na
6855	2800	0	22	44	0	0	0	na	na	na
6901	1728	8	0	0	0	0	0	na	na	na
6971	9138	0	58	32	32	4.45	0	na	na	na
6971	9203	0	48	32	0	0	0	na	na	na
7016	6781	0	7	0	0	0	0	na	na	na
7016	6792	14	0	0	0	0	0	na	na	na
7018	11211	0	33	33	33	16.39	7	7	T057E23	12.6cM
7018	11231	11	0	33	0	0	7	7	na	na
7018	11327	33	0	33	0	0	7	7	na	na
7062	501	26	0	26	0	0	0	na	na	na
7071	4952	31	0	31	0	0	0	na	na	na
7106	561	0	23	41	41	15.78	0	na	na	na
7106	563	41	0	41	0	0	0	na	na	na
7114	11562	30	0	30	0	0	0	na	na	na
7114	11621	0	7	30	30	16.44	0	na	na	na
7192	6977	0	24	16	16	19.5	25	25	T005M22	32.0cM
7192	7007	0	24	16	16	19.5	25	25	T005M22	32.0cM
7201	7626	33	0	0	0	0	0	na	na	na
7201	7631	0	35	0	0	0	0	na	na	na
7220	3154	0	71	0	0	0	0	na	na	na
7230	8500	0	33	33	33	2.94	0	na	na	na
7230	8550	33	0	33	0	0	0	na	na	na
7236	10908	0	10	0	0	0	0	na	na	na
7250	10067	0	10	0	0	0	0	na	na	na
7254	5511	0	3	14	0	0	0	9	na	na
7254	5533	0	3	14	0	0	0	22	na	na
7254	5534	0	3	14	0	0	0	22	na	na
7254	5540	0	3	14	0	0	0	22	na	na
7254	5547	0	3	14	0	0	0	22	na	na
7261	5686	0	10	7	7	24.54	13	13	T073B11	2.1cM
7261	5710	7	0	7	0	0	13	13	na	na
7264	1172	0	4	0	0	0	0	na	na	na
7286	9407	16	0	0	0	0	0	na	na	na
7359	6954	0	37	29	29	7.52	9	9	T046E07	26.1cM
7359	6957	0	37	29	29	7.52	9	9	T046E07	26.1cM
7372	2217	24	0	24	0	0	0	na	na	na
7372	2241	24	0	24	0	0	0	na	na	na
7372	2279	0	54	24	24	69.04	0	na	na	na
7399	1029	0	21	0	0	0	0	na	na	na
7399	1059	6	0	0	0	0	0	na	na	na
7410	11076	24	0	24	0	0	0	na	na	na
7422	2114	10	0	25	0	0	20	20	na	na
7422	2131	0	36	25	25	14.14	20	20	S013E03	32.3cM
7437	9969	1	0	0	0	0	0	na	na	na
7476	68	0	14	0	0	0	0	na	na	na
7502	4491	0	5	0	0	0	0	na	na	na
7511	10018	0	14	0	0	0	0	na	na	na
7519	3362	15	0	15	0	0	0	na	na	na
7519	3389	15	0	15	0	0	0	na	na	na
7560	2481	49	0	49	0	0	0	11	na	na
7567	10237	28	0	28	0	0	0	na	na	na
7567	10289	0	8	28	0	0	0	na	na	na
7570	837	0	61	49	49.2	0	0	16	T645F02	19.1cM
7575	3347	0	37	29	29	0	0	na	na	na
7616	4038	17	0	17	0	0	22	22	na	na
7616	4041	17	0	17	0	0	22	22	na	na
7637	9751	40	0	40	0	0	0	na	na	na
7637	9821	0	21	40	40	0	0	na	na	na
7655	2227	27	0	0	0	0	0	na	na	na
7655	2257	28	0	28	0	0	8	8	na	na
7680	8652	4	0	0	0	0	0	na	na	na
7680	8698	0	29	0	0	0	0	na	na	na
7682	2315	0	32	0	0	0	0	na	na	na
7682	2576	0	69	0	0	0	0	na	na	na
7683	322	25	0	25	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
7760	7860	0	37	29	29	5.35	0	na	na	na
7780	4522	23	0	23	0	0	11	11	na	na
7780	4570	0	26	23	23	0	0	11	T055C09	48.6cM
7780	8148	0	19	23	23.2	44.17	0	11	T055C09	48.6cM
7825	2346	0	1	0	0	0	0	na	na	na
7836	5294	45	0	45	0	0	0	na	na	na
7836	5299	45	0	45	0	0	0	na	na	na
7874	6087	34	0	34	0	0	4	4	na	na
7874	8284	0	5	34	34	2.63	4	4	T605A18	42.6cM
7881	4543	17	0	17	0	0	0	16	na	na
7881	4544	0	28	17	0	0	0	16	na	na
7881	4547	17	0	17	0	0	0	16	na	na
7911	6222	0	4	0	0	0	0	na	na	na
7949	8352	5	0	0	0	0	0	na	na	na
7962	3756	2	0	0	0	0	0	na	na	na
7962	3759	2	0	0	0	0	0	na	na	na
7984	1276	37	0	37	0	0	0	na	na	na
7984	1299	0	42	37	0	0	0	na	na	na
8031	8323	2	0	0	0	0	0	na	na	na
8035	8209	24	0	24	0	0	0	10	na	na
8035	8226	0	69	24	24	0	0	10	S006E22	9.0cM
8048	1921	0	58	32	32	25.36	24	24	T630A04	39.7cM
8063	7803	0	55	43	0	0	11	11	na	na
8063	7807	0	55	43	0	0	11	11	na	na
8063	7809	0	55	43	0	0	11	11	S079O18	61.9cM
8071	52	0	39	0	0	0	0	na	na	na
8093	6158	0	42	37	0	0	0	na	na	na
8093	6183	0	52	37	37	0	0	na	na	na
8093	6185	0	42	37	0	0	0	na	na	na
8098	6127	42	0	42	0	0	0	na	na	na
8124	2020	16	0	0	0	0	0	na	na	na
8135	2689	0	52	0	0	0	0	na	na	na
8152	6050	23	0	0	0	0	0	na	na	na
8155	857	5	0	0	0	0	0	na	na	na
8155	869	0	2	0	0	0	0	na	na	na
8156	7593	31	0	31	0	0	0	na	na	na
8178	9518	0	10	0	0	0	0	na	na	na
8189	5590	3	0	0	0	0	0	na	na	na
8189	5591	16	0	0	0	0	0	na	na	na
8200	9594	0	5	5	5	24.68	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
8200	9600	0	5	5	5	24.68	0	na	na	na
8200	9605	0	5	5	5	24.68	0	na	na	na
8209	2001	53	0	53	0	0	0	na	na	na
8209	2011	53	0	53	0	0	0	na	na	na
8209	2075	0	54	53	0	0	0	na	na	na
8224	8574	15	0	15	0	0	0	na	na	na
8231	1177	0	34	8	8	6.15	0	na	na	na
8262	3649	30	0	30	0	0	5	5	na	na
8267	7189	0	35	0	0	0	0	na	na	na
8267	7246	0	35	0	0	0	0	na	na	na
8276	1177	0	43	15	0	0	0	na	na	na
8276	1200	15	0	15	0	0	0	na	na	na
8311	5744	32	0	32	0	0	0	na	na	na
8315	9237	15	0	15	0	0	0	na	na	na
8321	5145	0	32	0	0	0	0	na	na	na
8325	3209	0	34	8	8	14.3	19	19	T631M06	22.8cM
8325	3258	8	0	8	0	0	19	19	na	na
8369	159	0	31	9	9	0	0	17	T619N03	0.0cM
8369	168	0	31	9	9	0	0	na	na	na
8369	169	0	31	9	9	0	17	na	na	na
8370	2602	0	29	0	0	0	0	na	na	na
8370	2623	0	20	0	0	0	0	na	na	na
8381	2027	0	9	0	0	0	0	na	na	na
8381	2041	0	9	0	0	0	0	na	na	na
8404	9075	7	0	0	0	0	0	na	na	na
8405	3739	11	0	0	0	0	0	na	na	na
8405	3749	11	0	0	0	0	0	na	na	na
8420	2080	15	0	15	0	0	0	na	na	na
8420	2094	15	0	15	0	0	0	na	na	na
8436	7954	0	19	23	23.2	21.29	0	na	na	na
8437	8245	1	0	0	0	0	0	na	na	na
8437	8374	15	0	15	0	0	0	na	na	na
8457	7388	8	0	0	0	0	0	na	na	na
8457	7405	8	0	0	0	0	0	na	na	na
8457	7441	0	25	0	0	0	0	na	na	na
8460	8331	0	11	0	0	0	0	na	na	na
8460	8340	0	11	0	0	0	0	na	na	na
8503	4510	0	4	0	0	0	0	na	na	na
8543	4122	0	28	0	0	0	0	na	na	na
8552	337	0	6	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
8552	346	0	6	0	0	0	0	na	na	na
8576	668	0	45	42	42	0	22	22	S001K13	53.7cM
8576	671	0	45	42	42	0	22	22	S001K13	53.7cM
8581	1126	0	2	0	0	0	0	na	na	na
8593	2972	7	0	0	0	0	0	na	na	na
8614	1806	0	21	0	0	0	0	na	na	na
8668	8603	0	18	0	0	0	0	na	na	na
8668	8651	0	18	0	0	0	0	na	na	na
8687	8622	0	8	28	0	0	0	na	na	na
8687	8651	0	8	28	0	0	0	na	na	na
8687	8653	28	0	28	0	0	0	na	na	na
8694	4635	0	49	13	13	36.69	0	na	na	na
8694	4664	13	0	13	0	0	0	na	na	na
8694	4665	0	13	13	0	0	0	na	na	na
8740	6928	0	49	13	13	25.26	18	18	T019J04	7.4cM
8740	6964	0	49	13	13	25.26	18	18	T019J04	7.4cM
8765	182	5	0	0	0	0	0	na	na	na
8794	3064	0	19	23	23.2	32.59	0	na	na	na
8794	3082	0	19	23	23.2	32.59	0	na	na	na
8803	203	0	34	8	8	12.12	0	na	na	na
8815	526	0	54	0	0	0	0	na	na	na
8818	4529	21	0	21	0	0	0	na	na	na
8825	6454	0	10	0	0	0	0	na	na	na
8837	651	0	43	15	0	0	0	na	na	na
8837	675	15	0	15	0	0	0	na	na	na
8837	7200	0	62	15	15	35.29	0	na	na	na
8837	7269	0	62	15	15	35.29	0	na	na	na
8852	1664	12	0	0	0	0	0	na	na	na
8852	1680	12	0	0	0	0	0	na	na	na
8893	2950	0	31	9	9	9.59	0	na	na	na
8893	2976	9	0	9	0	0	0	na	na	na
8893	2978	9	0	9	0	0	0	na	na	na
8893	2979	9	0	9	0	0	0	na	na	na
8911	8211	15	0	15	0	0	0	na	na	na
8911	8275	15	0	15	0	0	0	na	na	na
8932	545	0	27	0	0	0	0	na	na	na
8961	258	0	38	27	27	4.83	0	na	na	na
9034	2489	0	21	0	0	0	0	na	na	na
9034	2606	0	21	0	0	0	0	na	na	na
9034	2650	0	21	0	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
9076	5814	0	28	17	0	0	0	na	na	na
9076	5866	17	0	17	0	0	0	na	na	na
9076	5867	17	0	17	0	0	0	na	na	na
9094	7967	10	0	0	0	0	0	na	na	na
9118	6649	0	6	0	0	0	0	na	na	na
9146	7813	0	45	42	42	16.27	0	11	T024M06	23.8cM
9151	7079	0	8	28	0	0	8	8	na	na
9172	7922	0	41	45	0	0	0	na	na	na
9172	7952	0	40	45	45	28.9	0	na	na	na
9172	7977	0	40	45	45	28.9	0	na	na	na
9176	8017	11	0	0	0	0	0	na	na	na
9190	5877	5	0	0	0	0	0	na	na	na
9197	5958	0	13	0	0	0	0	na	na	na
9219	6779	0	13	0	0	0	0	na	na	na
9237	5892	0	14	0	0	0	0	na	na	na
9244	830	0	1	0	0	0	0	na	na	na
9244	833	0	1	0	0	0	0	na	na	na
9302	6688	0	68	49	49	0	0	23	T630K11	40.4cM
9326	1438	0	48	32	0	0	24	24	na	na
9335	2374	31	0	31	0	0	0	na	na	na
9335	2388	0	47	31	31	0	0	na	na	na
9394	3694	0	1	0	0	0	0	na	na	na
9394	3715	0	1	0	0	0	0	na	na	na
9394	3725	0	1	0	0	0	0	na	na	na
9409	1558	3	0	0	0	0	0	na	na	na
9447	682	0	25	0	0	0	0	na	na	na
9448	1242	0	32	0	0	0	0	na	na	na
9453	6687	0	38	27	27	0	0	na	na	na
9503	863	0	38	27	27	1.8	0	2	T087M18	33.2cM
9503	870	0	38	27	27	1.8	0	2	T087M18	33.2cM
9533	752	0	67	52	52.4	14.99	0	18	B535J11	31.1cM
9534	6845	0	2	0	0	0	0	na	na	na
9534	6858	0	2	0	0	0	0	na	na	na
9557	1522	0	17	0	0	0	0	na	na	na
9607	805	0	40	45	45	0	0	na	na	na
9607	846	0	41	45	0	0	0	na	na	na
9607	870	0	40	45	45	0	0	na	na	na
9607	872	0	40	45	45	0	0	na	na	na
9619	1163	27	0	0	0	0	0	na	na	na
9624	2702	0	15	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
9634	1147	0	21	0	0	0	0	na	na	na
9634	1173	0	27	0	0	0	0	na	na	na
9670	1750	0	20	4	0	0	0	na	na	na
9670	1752	4	0	4	0	0	0	na	na	na
9670	1756	4	0	4	0	0	0	na	na	na
9670	1766	4	0	4	0	0	0	na	na	na
9670	1773	4	0	4	0	0	0	na	na	na
9670	1782	4	0	4	0	0	0	na	na	na
9682	7537	0	39	0	0	0	0	na	na	na
9707	3104	0	56	50	50.2	6.26	11	11	T641D12	14.3cM
9707	3152	0	59	50	50	0	11	11	T641D12	14.3cM
9734	6883	0	28	0	0	0	0	na	na	na
9770	4446	0	42	0	0	0	0	na	na	na
9772	965	1	0	0	0	0	0	na	na	na
9823	731	0	76	27	0	0	0	na	na	na
9823	732	0	76	27	0	0	0	na	na	na
9823	733	0	76	27	0	0	0	na	na	na
9823	734	0	76	27	0	0	0	na	na	na
9838	6288	48	0	48	0	0	0	na	na	na
9838	6289	0	4	48	0	0	0	na	na	na
9872	1498	0	6	0	0	0	0	na	na	na
9872	1582	9	0	0	0	0	0	na	na	na
9909	3292	0	6	0	0	0	0	na	na	na
9981	7810	0	17	0	0	0	0	na	na	na
10002	7440	0	46	0	0	0	0	na	na	na
10103	2695	10	0	0	0	0	0	na	na	na
10130	4353	17	0	17	0	0	3	3	na	na
10145	4986	0	14	0	0	0	0	na	na	na
10172	3516	0	5	0	0	0	0	na	na	na
10180	336	0	10	0	0	0	0	na	na	na
10186	6128	0	2	0	0	0	0	na	na	na
10213	2629	11	0	0	0	0	0	na	na	na
10254	1308	16	0	0	0	0	0	na	na	na
10262	2573	0	12	0	0	0	0	na	na	na
10287	2066	0	39	0	0	0	0	na	na	na
10292	5766	0	5	0	0	0	0	na	na	na
10318	6128	36	0	36	0	0	0	na	na	na
10327	6280	0	17	0	0	0	0	na	na	na
10374	2562	0	12	26	0	0	0	na	na	na
10374	7142	0	12	26	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
10423	6958	0	15	10	0	0	0	na	na	na
10423	6959	0	15	10	0	0	0	na	na	na
10437	1955	0	4	48	0	0	0	na	na	na
10437	2008	0	4	48	0	0	0	na	na	na
10437	2009	0	4	48	0	0	0	na	na	na
10457	804	0	41	0	0	0	0	na	na	na
10519	7238	26	0	26	0	0	0	na	na	na
10519	7243	0	12	26	0	0	0	na	na	na
10519	7295	26	0	26	0	0	0	na	na	na
10575	6960	0	7	0	0	0	0	na	na	na
10575	6966	14	0	0	0	0	0	na	na	na
10595	6986	45	0	45	0	0	0	27	na	na
10658	306	9	0	0	0	0	0	na	na	na
10658	317	9	0	0	0	0	0	na	na	na
10745	537	0	35	0	0	0	0	na	na	na
10747	1291	9	0	0	0	0	0	na	na	na
10747	1306	0	6	0	0	0	0	na	na	na
10794	2384	20	0	20	0	0	0	na	na	na
10794	2531	0	53	20	20	17.84	0	na	na	na
10794	2560	39	0	20	0	0	0	na	na	na
10805	5828	0	24	0	0	0	0	na	na	na
10805	5831	0	11	0	0	0	0	na	na	na
10869	1773	9	0	9	0	0	0	11	na	na
10869	1831	0	31	9	9	9.59	0	7	B001F15	21.4cM
10906	5064	4	0	4	0	0	0	na	na	na
10906	5068	4	0	4	0	0	0	na	na	na
10906	5095	4	0	4	0	0	0	na	na	na
10906	5114	4	0	4	0	0	0	na	na	na
10938	3956	52	60	52	52.3	34.55	0	2	T087M18	33.2cM
10963	7411	0	73	0	0	0	0	na	na	na
10995	7001	0	61	49	49.2	4.45	0	11	T626P12	23.8cM
10995	7003	0	61	49	49.2	4.45	0	11	T626P12	23.8cM
10995	7048	0	61	49	49.2	4.45	0	11	T626P12	23.8cM
10995	7057	0	61	49	49.2	4.45	0	11	T626P12	23.8cM
11001	1465	0	23	0	0	0	0	na	na	na
11031	7071	0	1	0	0	0	0	na	na	na
11068	5989	0	18	0	0	0	0	na	na	na
11091	979	0	30	51	0	0	0	na	na	na
11091	1003	0	30	51	0	0	0	na	na	na
11091	1041	0	30	51	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
11181	486	1	0	0	0	0	0	na	na	na
11199	1376	0	75	0	0	0	0	na	na	na
11276	5225	46	0	46	0	0	0	na	na	na
11293	5691	0	54	24	24	72.62	28	28	S601I05	0.0cM
11293	5733	24	0	24	0	0	28	28	na	na
11310	152	0	38	27	27	1.8	2	2	T029I21	18.1cM
11317	892	0	1	0	0	0	0	na	na	na
11317	934	0	1	0	0	0	0	na	na	na
11361	6815	1	0	0	0	0	0	na	na	na
11387	1273	13	0	0	0	0	0	na	na	na
11528	6203	28	0	28	0	0	8	8	na	na
11538	2143	31	0	31	0	0	23	23	na	na
11538	3459	31	0	31	0	0	23	23	na	na
11589	346	0	4	0	0	0	0	na	na	na
11609	2598	5	0	0	0	0	0	na	na	na
11731	1967	0	59	50	50	9.4	0	23	T643E09	15.9cM
11750	871	0	49	13	13	25.26	0	na	na	na
11750	902	13	0	13	0	0	0	na	na	na
11843	6292	0	4	0	0	0	0	na	na	na
11846	204	0	35	0	0	0	0	na	na	na
11846	210	11	0	0	0	0	0	na	na	na
11887	6783	0	39	0	0	0	0	na	na	na
11933	7017	20	0	20	0	0	0	na	na	na
11933	7023	0	47	20	20	46.47	0	na	na	na
11958	6080	0	40	45	45	0	27	27	S092O09	7.0cM
11958	6560	0	40	45	45	0	27	27	S092O09	7.0cM
11961	1401	0	1	0	0	0	0	na	na	na
11961	4807	1	0	0	0	0	0	na	na	na
11961	5637	0	1	0	0	0	0	na	na	na
11961	5652	0	1	0	0	0	0	na	na	na
11969	4969	0	4	0	0	0	0	na	na	na
11969	4988	7	0	0	0	0	0	na	na	na
12006	21	0	23	0	0	0	0	na	na	na
12088	6259	43	0	0	0	0	0	na	na	na
12088	6284	43	0	0	0	0	0	na	na	na
12116	1061	0	35	0	0	0	0	na	na	na
12172	481	4	0	4	0	0	22	22	na	na
12172	517	0	17	4	0	0	0	28	na	na
12204	4748	0	14	0	0	0	0	na	na	na
12238	928	0	32	0	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
12238	970	0	54	0	0	0	0	na	na	na
12279	53	0	31	0	0	0	0	na	na	na
12315	5302	0	58	32	32	0	24	24	B504B24	4.8cM
12324	577	2	0	0	0	0	0	na	na	na
12324	656	2	0	0	0	0	0	na	na	na
12349	6630	23	0	23	0	0	0	na	na	na
12349	6634	0	26	23	23	0	0	na	na	na
12438	548	0	35	0	0	0	0	na	na	na
12470	1772	0	52	37	37	6.77	0	na	na	na
12470	1774	37	0	37	0	0	0	na	na	na
12470	1791	37	0	37	0	0	0	na	na	na
12498	5627	16	0	0	0	0	0	na	na	na
12559	6832	0	40	0	0	0	0	na	na	na
12590	3280	0	30	51	0	0	0	9	na	na
12590	3283	0	30	51	0	0	0	9	na	na
12738	6267	43	0	43	0	0	0	na	na	na
12738	6315	0	26	43	0	0	0	na	na	na
12738	6344	0	55	43	0	0	0	na	na	na
12764	95	0	55	43	0	0	0	na	na	na
12793	5762	0	25	46	0	0	0	23	na	na
12793	5795	0	20	46	0	0	0	23	na	na
12796	6337	0	9	0	0	0	0	na	na	na
12987	1607	12	0	0	0	0	0	na	na	na
13011	1056	9	0	0	0	0	0	na	na	na
13012	908	15	0	15	0	0	0	na	na	na
13013	300	0	14	31	31	48.47	0	23	T092D01	24.3cM
13013	302	31	0	31	0	0	23	na	na	na
13037	4275	0	8	28	0	0	0	8	na	na
13037	4283	0	8	28	0	0	0	8	na	na
13037	4300	0	8	28	0	0	0	8	na	na
13037	4910	0	8	28	0	0	0	8	na	na
13076	5802	0	70	52	52.2	0	0	na	na	na
13076	5803	0	70	52	52.2	0	0	na	na	na
13167	4184	5	0	0	0	0	0	na	na	na
13243	1595	0	17	12	12.1	0	0	na	na	na
13243	1618	12	0	12	0	0	0	na	na	na
13243	1619	12	0	12	0	0	0	na	na	na
13287	569	0	2	0	0	0	0	na	na	na
13356	1134	0	27	0	0	0	0	na	na	na
13356	1139	0	27	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
13372	954	0	20	0	0	0	0	na	na	na
13374	2087	0	27	0	0	0	0	na	na	na
13374	2119	0	27	0	0	0	0	na	na	na
13419	689	0	29	22	22	6.7	6	6	T603N13	20.8cM
13419	752	0	29	22	22	6.7	6	6	T603N13	20.8cM
13419	906	0	29	22	22	6.7	6	6	T603N13	20.8cM
13447	951	13	0	0	0	0	0	na	na	na
13484	5200	0	20	0	0	0	0	na	na	na
13532	2631	20	0	20	0	0	23	23	na	na
13532	2658	0	47	20	20	37.67	23	23	T025C09	24.3cM
13539	5845	0	9	0	0	0	0	na	na	na
13539	5860	2	0	0	0	0	0	na	na	na
13543	736	0	6	0	0	0	0	na	na	na
13570	767	13	0	13	0	0	0	6	na	na
13570	812	0	49	13	13	29.23	6	6	T065E05	40.7cM
13642	5346	0	18	19	0	0	0	na	na	na
13642	5364	19	0	19	0	0	0	na	na	na
13668	6022	36	0	36	0	0	0	14	na	na
13668	6034	0	21	36	36	9.77	0	14	T631N03	10.0cM
13680	4064	0	17	12	12.1	0	0	na	na	na
13680	4067	0	17	12	12.1	0	0	na	na	na
13680	4077	0	16	12	12.2	29.5	0	na	na	na
13680	4092	0	16	12	12.2	29.5	0	na	na	na
13728	1330	0	13	0	0	0	0	na	na	na
13757	3664	16	0	0	0	0	0	na	na	na
13757	3668	16	0	0	0	0	0	na	na	na
13757	3694	16	0	0	0	0	0	na	na	na
13757	3703	3	0	0	0	0	0	na	na	na
13757	3723	3	0	0	0	0	0	na	na	na
13763	5292	39	0	20	0	0	0	na	na	na
13763	5366	20	0	20	0	0	0	na	na	na
13763	5374	0	53	20	20	0	0	na	na	na
13763	5375	0	53	20	20	0	0	na	na	na
13781	625	3	0	0	0	0	0	na	na	na
13791	1998	22	0	22	0	0	6	6	na	na
13791	5393	22	0	22	0	0	0	na	na	na
13791	5417	22	0	22	0	0	0	na	na	na
13792	5915	14	0	0	0	0	0	na	na	na
13792	5924	0	3	0	0	0	0	na	na	na
13792	5926	0	7	0	0	0	0	na	na	na
13847	6056	0	28	0	0	0	0	na	na	na
13861	454	16	0	0	0	0	0	na	na	na
13960	4771	0	12	0	0	0	0	na	na	na
14050	5993	0	5	5	5	0	4	4	T007M07	47.3cM
14050	6005	0	5	5	5	0	4	4	T007M07	47.3cM
14050	6014	5	0	5	0	0	4	4	na	na
14173	5413	0	12	0	0	0	0	na	na	na
14396	5119	0	23	0	0	0	0	na	na	na
14428	1127	0	22	43	0	0	0	24	na	na
14428	1152	0	22	43	0	0	0	24	na	na
14428	1181	0	22	43	0	0	0	24	na	na
14452	4606	45	0	45	0	0	0	27	na	na
14464	26	11	0	0	0	0	0	na	na	na
14541	2437	10	0	0	0	0	0	na	na	na
14580	5669	0	6	0	0	0	0	na	na	na
14749	3657	31	0	31	0	0	23	23	na	na
14758	5375	0	15	0	0	0	0	na	na	na
14786	1115	0	52	37	37	13.99	0	1	T038N15	11.5cM
14786	1165	0	52	37	37	13.99	0	1	T038N15	11.5cM
14817	268	10	0	0	0	0	0	na	na	na
14924	4864	0	12	0	0	0	0	na	na	na
14963	2079	0	1	0	0	0	0	na	na	na
14963	2152	0	1	0	0	0	0	na	na	na
15127	100	0	35	0	0	0	0	na	na	na
15128	4152	0	32	0	0	0	0	na	na	na
15154	2464	33	0	33	0	0	7	7	na	na
15154	2466	0	33	33	33	9.56	7	7	T050B08	8.9cM
15154	2511	0	35	33	0	0	7	7	na	na
15154	2512	0	33	33	33	9.56	7	7	T050B08	8.9cM
15154	2539	11	0	33	0	0	7	7	na	na
15154	2543	0	33	33	33	9.56	7	7	T050B08	8.9cM
15178	4914	0	19	23	23.2	18.19	0	na	na	na
15178	4969	23	0	23	0	0	0	na	na	na
15178	4993	0	19	23	23.2	18.19	0	na	na	na
15178	5002	0	19	23	23.2	18.19	0	na	na	na
15198	1468	21	0	21	0	0	0	23	na	na
15233	4535	0	20	0	0	0	0	na	na	na
15286	4481	0	25	0	0	0	0	na	na	na
15292	2596	0	70	52	52.2	0	16	16	T095M09	2.6cM
15311	2616	30	0	30	0	0	0	16	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
15312	256	42	0	42	0	0	11	11	na	na
15363	4515	37	0	37	0	0	26	26	na	na
15363	4516	37	0	37	0	0	26	26	na	na
15363	4573	37	0	37	0	0	26	26	na	na
15372	3379	0	4	0	0	0	0	na	na	na
15388	1663	0	21	0	0	0	0	na	na	na
15537	2491	39	0	39	0	0	0	23	na	na
15537	2521	39	0	39	0	0	0	23	na	na
15537	2560	39	0	39	0	0	0	23	na	na
15549	2539	25	0	25	0	0	0	na	na	na
15549	2593	25	0	25	0	0	0	na	na	na
15622	592	0	9	0	0	0	0	na	na	na
15622	618	0	9	0	0	0	0	na	na	na
15631	2253	22	0	22	0	0	0	na	na	na
15724	1650	0	31	0	0	0	0	na	na	na
15775	1645	0	27	0	0	0	0	na	na	na
15775	1668	6	0	0	0	0	0	na	na	na
15775	1710	6	0	0	0	0	0	na	na	na
15826	3925	0	24	16	16	0	0	na	na	na
15826	3939	16	0	16	0	0	0	na	na	na
15826	3941	0	24	16	16	0	0	na	na	na
15826	3963	0	24	16	16	0	0	na	na	na
15826	3994	0	24	16	16	0	0	na	na	na
15826	4006	0	24	16	16	0	0	na	na	na
15871	3703	0	43	15	0	0	0	20	na	na
15891	425	0	32	0	0	0	0	na	na	na
15896	1432	34	0	34	0	0	14	14	na	na
15899	454	0	16	0	0	0	0	na	na	na
16021	3071	0	44	47	47	0	25	25	T073B14	8.2cM
16046	3078	0	55	43	0	0	0	11	na	na
16053	1214	0	26	23	23	0	0	na	na	na
16053	1288	0	19	23	23.2	0	0	na	na	na
16053	1296	0	19	23	23.2	0	0	na	na	na
16079	1093	0	6	0	0	0	0	na	na	na
16092	3642	0	24	0	0	0	0	na	na	na
16101	886	0	24	0	0	0	0	na	na	na
16104	1071	0	63	0	0	0	0	na	na	na
16105	3493	38	0	38	0	0	0	na	na	na
16159	1815	0	21	0	0	0	0	na	na	na
16188	2528	0	14	0	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
16195	1715	35	0	0	0	0	0	na	na	na
16196	2639	3	0	0	0	0	0	na	na	na
16196	2657	3	0	0	0	0	0	na	na	na
16237	2269	0	33	33	33	16.39	0	na	na	na
16261	3062	0	43	15	0	0	0	na	na	na
16305	2366	21	0	21	0	0	0	11	na	na
16305	2887	21	0	21	0	0	0	1	na	na
16305	2966	21	0	21	0	0	0	1	na	na
16317	1064	0	21	0	0	0	0	na	na	na
16317	1240	0	21	0	0	0	0	na	na	na
16318	796	0	30	0	0	0	0	na	na	na
16348	609	25	0	25	0	0	0	9	na	na
16348	635	25	0	25	0	0	0	22	na	na
16348	658	25	0	25	0	0	0	22	na	na
16348	685	10	0	25	0	0	0	22	na	na
16358	3418	0	62	15	15	25.96	0	na	na	na
16358	3427	0	62	15	15	25.96	0	na	na	na
16358	3440	0	62	15	15	25.96	0	na	na	na
16367	1184	14	0	0	0	0	0	na	na	na
16383	3459	24	0	24	0	0	0	na	na	na
16383	3526	24	0	24	0	0	0	na	na	na
16383	3539	24	0	24	0	0	0	na	na	na
16439	2706	0	64	24	24	29.96	28	28	T080L13	12.7cM
16441	2579	0	7	30	30	4.46	0	na	na	na
16441	2608	0	7	30	30	4.46	0	na	na	na
16441	2634	30	0	30	0	0	0	na	na	na
16441	2859	0	7	30	30	4.46	0	na	na	na
16504	2213	0	16	0	0	0	0	na	na	na
16610	2628	0	54	0	0	0	0	na	na	na
16667	1937	0	16	12	12.2	0	3	3	S063O07	34.2cM
16667	1949	0	16	12	12.2	0	3	3	S063O07	34.2cM
16667	1982	0	16	12	12.2	0	3	3	S063O07	34.2cM
16667	1995	0	16	12	12.2	0	3	3	S063O07	34.2cM
16722	862	0	15	10	0	0	0	na	na	na
16722	864	0	15	10	0	0	0	na	na	na
16792	2756	8	0	8	0	0	19	19	na	na
16792	2772	0	34	8	8	12.12	19	19	T058A02	10.9cM
16792	2784	8	0	8	0	0	19	19	na	na
16971	808	51	0	51	0	0	9	9	na	na
16971	865	51	0	51	0	0	9	9	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
16983	423	0	20	0	0	0	0	na	na	na
17006	3117	1	0	0	0	0	0	na	na	na
17009	2605	0	8	28	0	0	8	8	na	na
17013	1166	44	0	44	0	0	0	na	na	na
17040	2263	0	15	0	0	0	0	na	na	na
17067	1056	0	30	0	0	0	0	na	na	na
17072	2696	0	4	0	0	0	0	na	na	na
17082	1250	24	0	24	0	0	0	11	na	na
17115	370	0	18	0	0	0	0	na	na	na
17117	859	20	0	0	0	0	0	na	na	na
17127	1382	0	74	49	0	0	23	23	na	na
17127	1422	0	74	49	0	0	23	23	na	na
17127	1433	0	74	49	0	0	23	23	na	na
17145	1257	11	0	0	0	0	0	na	na	na
17145	1258	33	0	0	0	0	0	na	na	na
17150	2071	0	13	0	0	0	0	na	na	na
17167	1478	0	68	49	49	0	23	23	T618F04	51.5cM
17167	1490	0	68	49	49	0	23	23	B504B24	4.8cM
17167	1537	0	61	49	49.2	0	23	23	T618F04	51.5cM
17169	2214	0	25	0	0	0	0	na	na	na
17169	2230	8	0	0	0	0	0	na	na	na
17256	1316	0	56	50	50.2	18.97	3	3	T091P10	0.0cM
17256	1342	0	56	50	50.2	18.97	3	3	T091P10	0.0cM
17350	2428	20	0	0	0	0	0	na	na	na
17379	1997	0	21	0	0	0	0	na	na	na
17379	2023	6	0	0	0	0	0	na	na	na
17379	2026	0	21	0	0	0	0	na	na	na
17379	2103	6	0	0	0	0	0	na	na	na
17468	702	0	7	30	30.2	0	0	10	T622J09	10.6cM
17468	711	0	7	30	30.2	0	0	10	T622J09	10.6cM
17468	716	0	7	30	30.2	0	0	10	T622J09	10.6cM
17468	730	0	7	30	30.2	0	0	10	T622J09	10.6cM
17471	1151	22	0	22	0	0	6	6	na	na
17540	989	0	30	0	0	0	0	na	na	na
17540	1067	0	30	0	0	0	0	na	na	na
17576	338	0	48	32	0	0	0	na	na	na
17609	708	9	0	0	0	0	0	na	na	na
17620	1043	34	0	34	0	0	0	na	na	na
17620	1059	34	0	34	0	0	0	na	na	na
17646	2678	35	0	0	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
17716	1906	0	27	0	0	0	0	na	na	na
17722	785	26	0	26	0	0	21	21	na	na
17722	816	0	39	26	26	16.94	21	21	T620E17	42.3cM
17735	2788	36	0	36	0	0	14	14	na	na
17796	200	0	12	0	0	0	0	na	na	na
17805	2313	0	14	0	0	0	0	na	na	na
17880	2911	3	0	0	0	0	0	na	na	na
17880	2923	3	0	0	0	0	0	na	na	na
17919	2223	23	0	0	0	0	0	na	na	na
17938	1951	46	0	46	0	0	0	na	na	na
18012	1473	0	32	0	0	0	0	na	na	na
18083	624	46	0	46	0	0	0	na	na	na
18083	650	0	25	46	0	0	0	na	na	na
18083	658	46	0	46	0	0	0	na	na	na
18168	2025	0	17	0	0	0	0	na	na	na
18231	170	18	0	18	0	0	13	13	na	na
18231	1875	18	0	18	0	0	13	13	na	na
18262	2532	0	37	29	29	7.52	10	10	T640P13	30.1cM
18372	1671	36	0	36	0	0	0	2	na	na
18372	1701	36	0	36	0	0	22	22	na	na
18372	1705	0	21	36	36	0	0	2	T628H14	0.0cM
18372	1783	36	0	36	0	0	0	2	na	na
18372	1832	0	21	36	36	2.22	0	20	T093D18	21.3cM
18372	1840	0	21	36	36	2.22	0	20	T093D18	21.3cM
18374	488	0	17	12	12.1	0	3	3	S081O09	23.6cM
18374	506	12	0	12	0	0	0	3	na	na
18374	510	0	17	12	12.1	0	0	3	S081O09	23.6cM
18374	511	0	16	12	12.2	11.51	0	3	S081O09	23.6cM
18407	459	10	0	10	0	0	0	na	na	na
18407	495	0	15	10	0	0	0	na	na	na
18446	1002	52	60	52	52	0	25	25	T005M22	32.0cM
18607	1564	0	4	0	0	0	0	na	na	na
18607	1565	0	4	0	0	0	0	na	na	na
18635	2819	32	0	32	0	0	0	na	na	na
18635	2842	0	48	32	0	0	0	na	na	na
18635	2848	0	48	32	0	0	0	na	na	na
18694	305	35	0	0	0	0	0	na	na	na
18712	347	0	40	0	0	0	0	na	na	na
18788	440	0	40	0	0	0	0	na	na	na
18852	1407	0	63	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
18875	1402	0	42	0	0	0	0	na	na	na
18878	2311	13	0	0	0	0	0	na	na	na
18956	1807	0	9	41	41	82.43	0	1	T620I07	27.8cM
18956	1849	41	0	41	0	0	0	1	na	na
19029	84	4	0	0	0	0	0	na	na	na
19137	833	8	0	0	0	0	0	na	na	na
19380	2706	6	0	0	0	0	0	na	na	na
19380	2727	0	21	0	0	0	0	na	na	na
19470	2720	0	21	0	0	0	0	na	na	na
19570	1957	13	0	0	0	0	0	na	na	na
19570	1996	13	0	0	0	0	0	na	na	na
19599	1156	6	0	0	0	0	0	na	na	na
19599	1162	6	0	0	0	0	0	na	na	na
19632	603	10	0	0	0	0	0	na	na	na
19728	1276	0	32	24	0	0	0	na	na	na
19728	1282	0	64	24	24	29.96	0	na	na	na
19728	1314	0	64	24	24	29.96	0	na	na	na
19742	1286	0	35	0	0	0	0	na	na	na
19787	1787	1	0	0	0	0	0	na	na	na
19867	1311	28	0	28	0	0	8	8	na	na
19873	457	0	22	43	0	0	0	3	na	na
19873	494	0	22	43	0	0	0	na	na	na
19873	495	0	22	43	0	0	0	na	na	na
19873	500	0	22	43	0	0	12	na	na	na
19873	511	0	22	43	0	0	0	3	na	na
19873	524	0	45	43	0	0	0	3	na	na
19873	533	0	22	43	0	0	0	3	na	na
19894	1138	0	2	34	0	0	0	na	na	na
19894	1148	34	0	34	0	0	0	na	na	na
19894	1157	0	5	34	34	0	0	na	na	na
19894	1194	0	2	34	0	0	0	na	na	na
19910	1345	0	56	50	50.2	16.31	0	na	na	na
19910	1365	0	56	50	50.2	16.31	0	na	na	na
19934	1959	0	5	0	0	0	0	na	na	na
19934	1960	0	2	0	0	0	0	na	na	na
19955	1972	0	8	28	0	0	8	8	na	na
20030	1643	13	0	0	0	0	0	na	na	na
20030	1691	13	0	0	0	0	0	na	na	na
20062	653	0	33	33	33	0	7	7	T053N08	0.0cM
20078	168	0	19	23	23.2	11.96	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
20078	177	0	19	23	23.2	11.96	0	na	na	na
20092	2514	0	11	0	0	0	0	na	na	na
20308	1024	5	0	0	0	0	0	na	na	na
20342	263	0	69	0	0	0	0	na	na	na
20356	2270	0	32	0	0	0	0	na	na	na
20397	152	0	9	0	0	0	0	na	na	na
20404	401	13	0	0	0	0	0	na	na	na
20436	378	0	22	0	0	0	0	na	na	na
20436	432	0	22	0	0	0	0	na	na	na
20557	1803	8	0	0	0	0	0	na	na	na
20595	158	0	11	0	0	0	0	na	na	na
20634	1138	0	8	28	0	0	0	na	na	na
20663	714	0	50	11	11	11.5	5	5	S002C24	10.1cM
20818	560	0	22	0	0	0	0	na	na	na
20818	561	0	22	0	0	0	0	na	na	na
20934	636	30	0	30	0	0	0	1	na	na
21007	688	0	5	34	34	11.96	0	na	na	na
21007	943	34	0	34	0	0	0	na	na	na
21039	985	41	0	41	0	0	0	na	na	na
21039	989	41	0	41	0	0	0	na	na	na
21039	990	2	0	41	0	0	0	na	na	na
21039	997	0	23	41	41	0	0	na	na	na
21056	191	9	0	0	0	0	0	na	na	na
21056	261	9	0	0	0	0	0	na	na	na
21197	1443	0	9	0	0	0	0	na	na	na
21260	1754	3	0	0	0	0	0	na	na	na
21269	395	0	47	0	0	0	0	na	na	na
21281	1132	25	0	25	0	0	0	na	na	na
21318	785	20	0	20	0	0	0	15	na	na
21318	789	0	47	20	20	42.12	0	15	T624A24	5.3cM
21318	801	20	0	20	0	0	0	15	na	na
21387	1316	32	0	32	0	0	0	na	na	na
21392	1354	0	7	0	0	0	0	na	na	na
21472	2246	0	2	0	0	0	0	na	na	na
21472	2267	0	2	0	0	0	0	na	na	na
21472	2313	0	2	0	0	0	0	na	na	na
21477	1134	0	25	0	0	0	0	na	na	na
21477	1138	0	25	0	0	0	0	na	na	na
21642	1360	0	70	52	52.2	0	16	16	T042H15	2.1cM
21688	130	0	9	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
21737	1490	0	13	38	0	0	0	na	na	na
21737	1491	0	13	38	0	0	0	na	na	na
21772	247	0	41	0	0	0	0	na	na	na
21772	248	0	41	0	0	0	0	na	na	na
21772	265	0	40	0	0	0	0	na	na	na
21872	1572	0	10	0	0	0	0	na	na	na
22122	1520	10	0	10	0	0	0	na	na	na
22122	1526	10	0	10	0	0	0	na	na	na
22122	1530	10	0	10	0	0	0	na	na	na
22122	1534	10	0	10	0	0	0	na	na	na
22444	570	0	39	26	26	9.33	21	21	T035I24	0.0cM
22444	585	0	12	26	0	0	21	21	na	na
22476	880	0	69	0	0	0	0	na	na	na
22686	1336	3	0	0	0	0	0	na	na	na
22830	137	0	59	50	50	0	11	11	T065P18	18.6cM
22831	2169	0	4	0	0	0	0	na	na	na
22831	2180	0	4	0	0	0	0	na	na	na
22891	510	0	11	0	0	0	0	na	na	na
22964	1845	0	57	0	0	0	0	na	na	na
23154	1892	0	10	0	0	0	0	na	na	na
23154	1944	0	4	0	0	0	0	na	na	na
23154	1946	0	10	0	0	0	0	na	na	na
23272	2076	0	75	0	0	0	0	na	na	na
23272	2077	0	75	0	0	0	0	na	na	na
23351	2123	2	0	0	0	0	0	na	na	na
23361	147	0	40	45	45	28.9	0	27	S082K03	39.6cM
23361	167	0	40	45	45	28.9	1	na	na	na
23442	2207	33	0	0	0	0	0	na	na	na
23460	1420	0	1	0	0	0	0	na	na	na
23503	1897	25	0	25	0	0	0	na	na	na
23549	1882	0	5	0	0	0	0	na	na	na
23549	1886	0	5	0	0	0	0	na	na	na
23549	1902	0	5	0	0	0	0	na	na	na
23549	1916	0	2	0	0	0	0	na	na	na
23549	1938	0	2	0	0	0	0	na	na	na
23628	2314	0	62	15	15	17.65	0	na	na	na
23663	248	17	0	17	0	0	0	na	na	na
23799	1937	27	0	27	0	0	0	na	na	na
23799	1944	27	0	27	0	0	0	na	na	na
23799	1994	27	0	27	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
23829	977	15	0	15	0	0	0	na	na	na
23943	1799	29	0	29	0	0	9	9	na	na
23965	1073	0	77	27	0	0	0	na	na	na
23965	1108	0	77	27	0	0	0	na	na	na
23965	1110	0	77	27	0	0	0	na	na	na
23965	1113	0	77	27	0	0	0	na	na	na
23998	248	0	2	0	0	0	0	na	na	na
24077	2262	16	0	0	0	0	0	na	na	na
24213	928	0	59	50	50	0	24	24	S080P17	8.4cM
24222	1018	0	20	0	0	0	0	na	na	na
24399	40	0	41	0	0	0	0	na	na	na
24399	85	0	41	0	0	0	0	na	na	na
24582	1588	0	21	0	0	0	0	na	na	na
24584	1473	0	19	23	23.2	0	0	na	na	na
24723	1696	0	16	12	12.2	0	0	na	na	na
24723	1731	0	16	12	12.2	0	0	na	na	na
24760	503	27	0	0	0	0	0	na	na	na
24857	1859	0	26	0	0	0	0	na	na	na
24945	1546	1	0	0	0	0	0	na	na	na
24968	1534	0	57	0	0	0	0	na	na	na
25122	828	30	0	30	0	0	0	na	na	na
25122	855	0	3	30	30.2	42.36	0	na	na	na
25142	877	0	25	0	0	0	0	na	na	na
25142	886	0	25	0	0	0	0	na	na	na
25216	1527	3	0	0	0	0	0	na	na	na
25284	721	15	0	15	0	0	0	na	na	na
25321	1503	48	0	48	0	0	0	19	na	na
25324	1704	0	2	0	0	0	0	na	na	na
25405	1964	20	0	0	0	0	0	na	na	na
25419	664	0	37	29	29	7.52	0	na	na	na
25612	1288	20	0	20	0	0	0	na	na	na
25612	1355	0	53	20	20	7.51	0	na	na	na
25612	1422	20	0	20	0	0	0	na	na	na
25612	1431	0	14	20	0	0	0	na	na	na
25884	1216	0	25	0	0	0	0	na	na	na
25903	764	0	18	0	0	0	0	na	na	na
25995	1648	28	0	28	0	0	0	na	na	na
26000	2072	0	73	0	0	0	0	na	na	na
26126	1121	0	2	0	0	0	0	na	na	na
26126	1159	0	2	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
26126	1200	5	0	0	0	0	0	na	na	na
26140	990	0	9	0	0	0	0	na	na	na
26141	1164	0	25	46	0	0	0	na	na	na
26141	1222	46	0	46	0	0	0	na	na	na
26269	1920	7	0	0	0	0	0	na	na	na
26542	2005	34	0	34	0	0	4	4	na	na
26542	2009	34	0	34	0	0	4	4	na	na
26658	992	0	30	0	0	0	0	na	na	na
26658	1041	0	30	0	0	0	0	na	na	na
26709	1758	0	7	0	0	0	0	na	na	na
26709	1771	0	7	0	0	0	0	na	na	na
26744	1196	35	0	35	0	0	0	20	na	na
26744	1243	0	6	35	35	5.27	0	20	S078G22	44.2cM
26744	1244	0	6	35	35	5.27	0	20	S078G22	44.2cM
26903	461	0	29	22	22	6.7	0	na	na	na
26903	466	0	20	22	0	0	0	na	na	na
26903	493	0	29	22	22	6.7	0	na	na	na
26944	878	45	0	45	0	0	27	27	na	na
26955	161	39	0	0	0	0	0	na	na	na
26955	242	39	0	0	0	0	0	na	na	na
26955	281	20	0	0	0	0	0	na	na	na
27027	1718	0	21	0	0	0	0	na	na	na
27119	1715	0	1	0	0	0	0	na	na	na
27119	1736	0	18	0	0	0	0	na	na	na
27168	191	48	0	48	0	0	0	13	na	na
27168	196	7	0	48	0	0	0	na	na	na
27168	212	48	0	48	0	0	0	na	na	na
27168	230	48	0	48	0	0	0	na	na	na
27168	271	0	4	48	0	0	13	na	na	na
27237	2026	0	44	47	47	6.83	0	na	na	na
27237	2028	16	0	47	0	0	0	na	na	na
27237	2040	0	44	47	47	6.83	0	na	na	na
27237	2060	47	0	47	0	0	0	na	na	na
27314	112	20	0	0	0	0	0	na	na	na
27389	1183	0	31	0	0	0	0	na	na	na
27435	46	0	11	0	0	0	0	na	na	na
27503	2051	17	0	17	0	0	0	na	na	na
27582	1578	21	0	21	0	0	11	11	na	na
27638	1608	0	22	0	0	0	0	na	na	na
27670	1429	0	12	0	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
27714	1054	10	0	0	0	0	0	na	na	na
27902	676	0	10	0	0	0	0	na	na	na
27902	688	0	10	0	0	0	0	na	na	na
27983	808	0	55	43	0	0	0	na	na	na
27983	851	0	55	43	0	0	0	na	na	na
27990	235	0	29	0	0	0	0	na	na	na
28090	1731	0	7	30	30	16.44	15	15	T046N24	19.2cM
28090	1782	30	0	30	0	0	15	15	na	na
28305	1283	0	8	28	0	0	0	na	na	na
28331	1092	0	28	17	0	0	0	na	na	na
28331	1095	0	28	17	0	0	0	na	na	na
28335	197	17	0	17	0	0	0	na	na	na
28335	241	0	28	17	0	0	0	na	na	na
28357	661	14	0	0	0	0	0	na	na	na
28357	739	0	7	0	0	0	0	na	na	na
28357	841	0	3	0	0	0	0	na	na	na
28423	1611	39	0	0	0	0	0	na	na	na
28423	1675	0	14	0	0	0	0	na	na	na
28524	494	8	0	0	0	0	0	na	na	na
28720	1949	0	38	27	27	1.8	0	na	na	na
28720	2005	0	38	27	27	1.8	0	na	na	na
28720	2006	0	38	27	27	1.8	0	na	na	na
28762	1406	23	0	0	0	0	0	na	na	na
28814	1343	0	3	14	0	0	25	25	na	na
28814	1346	0	3	14	0	0	0	25	na	na
28814	1366	0	3	14	0	0	0	25	na	na
28814	1589	0	3	14	0	0	0	25	na	na
28814	1993	0	3	14	0	0	25	25	na	na
28814	2057	0	3	14	0	0	25	25	na	na
28885	977	0	20	0	0	0	0	na	na	na
28885	1029	4	0	0	0	0	0	na	na	na
29002	277	9	0	0	0	0	0	na	na	na
29054	1731	0	58	32	32	0	0	na	na	na
29054	1758	0	58	32	32	0	0	na	na	na
29139	220	32	0	32	0	0	0	na	na	na
29474	240	6	0	0	0	0	0	na	na	na
29474	695	6	0	0	0	0	0	na	na	na
29525	1339	21	0	21	0	0	0	na	na	na
29525	1341	21	0	21	0	0	0	na	na	na
29525	1343	21	0	21	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-1: (continued)

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
29603	523	19	0	19	0	0	0	na	na	na
29679	134	0	8	28	0	0	8	8	na	na
29791	890	0	9	0	0	0	0	na	na	na
29794	678	0	4	0	0	0	0	na	na	na
29794	691	7	0	0	0	0	0	na	na	na
29840	1696	0	23	0	0	0	0	na	na	na
29945	270	19	0	19	0	0	0	na	na	na
29945	279	19	0	19	0	0	0	na	na	na
29945	315	1	0	19	0	0	0	na	na	na

Scaf	Pos	MLG	PLG	LOD1	Ord	Dist	Uchr	Tchr	BLoc	BPos
29995	360	0	4	0	0	0	0	na	na	na
30158	311	17	0	17	0	0	22	22	na	na
30319	1272	0	22	0	0	0	0	na	na	na
30347	684	5	0	0	0	0	0	na	na	na
30347	725	0	5	0	0	0	0	na	na	na
30468	1393	0	49	13	13	44.49	0	na	na	na
30479	1491	0	3	0	0	0	0	na	na	na
30495	1543	0	53	0	0	0	0	na	na	na

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffold, MLG and PLG are the maternal and paternal linkage group assignment, LOD1 are the assignments with LOD score>1, Ord identifies ordered loci and Dist gives their map location, Uchr and Tchr give the identify of unique and top tblastx hit in *B. mori* chromosomes, Bloc and BPos indicate the position of mapped orthologous *B. mori* markers.

Appendix 6-2: UniRef90 blastx results

Scaf	Pos	UniRef Hit	%id	Id	Len	Gap	Evalue	Bit
5	243950	UniRef90_G6CV02 Putative Y71H2B.5 n=1 Tax=Danaus plexippus RepID=G6CV02_DANPL	77.98	85	109	0	9.00E-88	184
5	243952	UniRef90_G6CV02 Putative Y71H2B.5 n=1 Tax=Danaus plexippus RepID=G6CV02_DANPL	77.98	85	109	0	4.00E-87	184
5	244000	UniRef90_G6CV02 Putative Y71H2B.5 n=1 Tax=Danaus plexippus RepID=G6CV02_DANPL	77.98	85	109	0	2.00E-77	184
17	62543	UniRef90_S4PC35 Putative RNA-binding protein 19 (Fragment) n=1 Tax=Pararge aegeria RepID=S4PC35_9NEOP	76.25	61	80	0	2.00E-34	140
35	26958	UniRef90_G6CW84 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6CW84_DANPL	98.27	170	173	0	3.00E-109	353
43	109427	UniRef90_A4KWF4 Reverse transcriptase n=1 Tax=Ostrinia nubilalis RepID=A4KWF4_OSTNU	79.75	63	79	0	2.00E-22	104
43	109452	UniRef90_A4KWG0 Reverse transcriptase n=2 Tax=Ostrinia nubilalis RepID=A4KWG0_OSTNU	79.75	63	79	0	2.00E-23	105
43	109462	UniRef90_A4KWG0 Reverse transcriptase n=2 Tax=Ostrinia nubilalis RepID=A4KWG0_OSTNU	79.75	63	79	0	5.00E-24	105
45	98147	UniRef90_G6DRW2 Endonuclease-reverse transcriptase n=1 Tax=Danaus plexippus RepID=G6DRW2_DANPL	56.76	42	74	4	3.00E-12	60.8
47	1387	UniRef90_UPI000350257A PREDICTED: ribonuclease 3-like n=1 Tax=Bombyx mori RepID=UPI000350257A	76.92	50	65	1	3.00E-25	116
47	41640	UniRef90_G6CUP6 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6CUP6_DANPL	74.67	112	150	2	2.00E-84	181
47	41655	UniRef90_G6CUP6 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6CUP6_DANPL	73.61	106	144	2	4.00E-78	169
48	91492	UniRef90_H9ISD2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9ISD2_BOMMO	52.84	93	176	0	5.00E-53	187
48	91558	UniRef90_H9JAL9 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JAL9_BOMMO	60.78	93	153	0	1.00E-57	195
51	55498	UniRef90_H9JNG8 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JNG8_BOMMO	84.48	49	58	0	6.00E-23	106
52	93912	UniRef90_D7F171 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F171_BOMMO	72.73	88	121	0	3.00E-46	177
52	93924	UniRef90_D7F171 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F171_BOMMO	72.73	88	121	0	3.00E-46	177
52	93995	UniRef90_D7F171 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F171_BOMMO	72.73	88	121	0	3.00E-46	177
52	108645	UniRef90_D7F164 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F164_BOMMO	39.69	52	131	5	3.00E-13	79.3
53	91095	UniRef90_G6DST8 Chromodomain-helicase-DNA-binding protein 1 n=1 Tax=Danaus plexippus RepID=G6DST8_DANPL	80	64	80	1	4.00E-14	82
53	114919	UniRef90_H9J1W1 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9J1W1_BOMMO	91.67	66	72	0	4.00E-33	139
53	114922	UniRef90_H9J1W1 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9J1W1_BOMMO	91.67	66	72	0	4.00E-33	139
56	14890	UniRef90_D1LU95 Mutant cadherin n=1 Tax=Helicoverpa armigera RepID=D1LU95_HELAM	47.54	58	122	1	3.00E-23	109
56	14916	UniRef90_D1LU95 Mutant cadherin n=1 Tax=Helicoverpa armigera RepID=D1LU95_HELAM	46.72	57	122	1	3.00E-22	107
56	129718	UniRef90_Q9BLI5 TRAS3 protein n=1 Tax=Bombyx mori RepID=Q9BLI5_BOMMO	64.52	120	186	0	4.00E-84	264
56	129771	UniRef90_Q9BLI5 TRAS3 protein n=1 Tax=Bombyx mori RepID=Q9BLI5_BOMMO	65.52	133	203	0	7.00E-92	289
86	75002	UniRef90_H9IZJ2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9IZJ2_BOMMO	85.81	133	155	1	4.00E-49	172
86	75023	UniRef90_H9IZJ2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9IZJ2_BOMMO	85.81	133	155	1	4.00E-49	172
86	75077	UniRef90_H9IZJ2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9IZJ2_BOMMO	85.81	133	155	1	4.00E-49	172
93	119037	UniRef90_H9J5A9 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9J5A9_BOMMO	91.49	43	47	0	3.00E-20	99.8
110	114431	UniRef90_G6DRW2 Endonuclease-reverse transcriptase n=1 Tax=Danaus plexippus RepID=G6DRW2_DANPL	67.62	71	105	2	3.00E-28	117
133	24433	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	78.46	51	65	1	2.00E-20	97.8
133	24463	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	78.21	61	78	2	4.00E-26	114
133	24466	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	78.48	62	79	2	1.00E-26	115
133	24473	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	79.01	64	81	2	1.00E-27	119
133	24483	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	80.25	65	81	2	3.00E-28	120
133	24486	UniRef90_H9IVK6 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IVK6_BOMMO	79.27	65	82	2	2.00E-28	120
139	34840	UniRef90_G6D3G9 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6D3G9_DANPL	65.06	54	83	3	4.00E-19	95.1
139	34846	UniRef90_G6D3G9 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6D3G9_DANPL	65.06	54	83	3	4.00E-19	95.1

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffolds, UniRef Hit is the top blastx hit UniRef90 protein, %id is the percentage of identical base matches between the locus and UniRef90 protein match, Id is the number of identical base matches, Len is the alignment length, Gap indicates the number of gap extensions in the alignment, evalue is the significance of the blastx hit, and Bit is the bit score of the hit.

Appendix 6-2: (continued)

Scaf	Pos	UniRef Hit	%id	Id	Len	Gap	Evalue	Bit
139	34901	UniRef90_G6D3G9 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6D3G9_DANPL	65.06	54	83	3	4.00E-19	95.1
140	14224	UniRef90_H9J349 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9J349_BOMMO	54.43	43	79	1	1.00E-19	91.3
140	14263	UniRef90_H9J349 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9J349_BOMMO	54.43	43	79	1	1.00E-19	91.3
143	22035	UniRef90_G6D0W2 Putative Bbs2 protein n=1 Tax=Danaus plexippus RepID=G6D0W2_DANPL	57.14	44	77	2	4.00E-15	84.7
143	22041	UniRef90_G6D0W2 Putative Bbs2 protein n=1 Tax=Danaus plexippus RepID=G6D0W2_DANPL	57.14	44	77	2	4.00E-15	84.7
143	46893	UniRef90_S4P9E2 Disc proliferation abnormal n=1 Tax=Pararge aegeria RepID=S4P9E2_9NEOP	83.78	62	74	1	8.00E-28	122
159	50179	UniRef90_H9IVZ7 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9IVZ7_BOMMO	97.92	47	48	0	4.00E-20	99.8
164	75962	UniRef90_D7F167 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F167_BOMMO	77.23	78	101	0	3.00E-41	163
164	75979	UniRef90_D7F167 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F167_BOMMO	77.23	78	101	0	3.00E-41	163
164	76029	UniRef90_D7F167 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D7F167_BOMMO	77.23	78	101	0	3.00E-41	163
172	991	UniRef90_H9JAA8 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JAA8_BOMMO	48	36	75	1	1.00E-11	68.9
172	994	UniRef90_H9JAA8 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JAA8_BOMMO	48	36	75	1	1.00E-11	68.9
172	1059	UniRef90_H9JAA8 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JAA8_BOMMO	48	36	75	1	1.00E-11	68.9
202	83905	UniRef90_G6DT96 Putative jumonji domain containing 1A n=1 Tax=Danaus plexippus RepID=G6DT96_DANPL	66.44	196	295	6	3.00E-65	216
217	5446	UniRef90_H9JLG2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JLG2_BOMMO	97.65	83	85	0	4.00E-44	173
217	28473	UniRef90_H9JLG2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JLG2_BOMMO	50	50	100	1	5.00E-17	91.3
217	28535	UniRef90_H9JLG2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JLG2_BOMMO	50	50	100	1	5.00E-17	91.3
217	28537	UniRef90_H9JLG2 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JLG2_BOMMO	50	50	100	1	5.00E-17	91.3
217	68948	UniRef90_B2CMB5 Phosphorylase n=6 Tax=Obtectomera RepID=B2CMB5_BOMMO	96.23	51	53	0	8.00E-24	111
217	69031	UniRef90_B2CMB5 Phosphorylase n=6 Tax=Obtectomera RepID=B2CMB5_BOMMO	96.23	51	53	0	8.00E-24	111
226	28078	UniRef90_G6DFP7 Reverse transcriptase n=1 Tax=Danaus plexippus RepID=G6DFP7_DANPL	63.33	38	60	0	4.00E-43	82.4
226	28119	UniRef90_G6DFP7 Reverse transcriptase n=1 Tax=Danaus plexippus RepID=G6DFP7_DANPL	63.33	38	60	0	4.00E-43	82.4
226	28137	UniRef90_G6DFP7 Reverse transcriptase n=1 Tax=Danaus plexippus RepID=G6DFP7_DANPL	63.33	38	60	0	5.00E-43	82.4
232	57456	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57475	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57482	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57487	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57517	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57518	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
232	57538	UniRef90_S4PN69 Adenosine kinase (Fragment) n=1 Tax=Pararge aegeria RepID=S4PN69_9NEOP	79.37	50	63	0	1.00E-24	107
283	73984	UniRef90_H9JHD1 Uncharacterized protein n=2 Tax=Obtectomera RepID=H9JHD1_BOMMO	97.73	43	44	0	9.00E-21	96.3
309	31041	UniRef90_H9IYS7 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9IYS7_BOMMO	67.09	106	158	1	2.00E-51	194
318	47617	UniRef90_D5LB38 Endonuclease-reverse transcriptase n=1 Tax=Bombyx mori RepID=D5LB38_BOMMO	58.99	105	178	2	1.00E-59	214
319	57362	UniRef90_S4PEW2 Nuclear protein localization (Fragment) n=1 Tax=Pararge aegeria RepID=S4PEW2_9NEOP	71.19	42	59	1	1.00E-19	94
336	29733	UniRef90_E2BIH4 GTP-binding nuclear protein Ran n=23 Tax=Pancrustacea RepID=E2BIH4_HARSA	95.71	134	140	0	4.00E-93	287
336	29740	UniRef90_E2BIH4 GTP-binding nuclear protein Ran n=23 Tax=Pancrustacea RepID=E2BIH4_HARSA	95.71	134	140	0	5.00E-93	286
344	5873	UniRef90_UPI00034FB1F9 PREDICTED: spectrin beta chain-like n=1 Tax=Bombyx mori RepID=UPI00034FB1F9	78.87	56	71	0	1.00E-26	120
344	5878	UniRef90_UPI00034FB1F9 PREDICTED: spectrin beta chain-like n=1 Tax=Bombyx mori RepID=UPI00034FB1F9	78.87	56	71	0	1.00E-26	120
344	18410	UniRef90_G6D2S7 Putative Spectrin beta chain n=1 Tax=Danaus plexippus RepID=G6D2S7_DANPL	88.16	67	76	0	2.00E-31	135

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffolds, UniRef Hit is the top blastx hit UniRef90 protein, %id is the percentage of identical base matches between the locus and UniRef90 protein match, Id is the number of identical base matches, Len is the alignment length, Gap indicates the number of gap extensions in the alignment, evalue is the significance of the blastx hit, and Bit is the bit score of the hit.

Appendix 6-2: (continued)

Scaf	Pos	UniRef Hit	%id	Id	Len	Gap	Evalue	Bit
344	24833	UniRef90_G6D2S7 Putative Spectrin beta chain n=1 Tax=Danaus plexippus RepID=G6D2S7_DANPL	89.71	61	68	0	2.00E-18	95.5
344	24841	UniRef90_G6D2S7 Putative Spectrin beta chain n=1 Tax=Danaus plexippus RepID=G6D2S7_DANPL	89.71	61	68	0	2.00E-18	95.5
411	39917	UniRef90_G6DE33 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6DE33_DANPL	88.26	218	247	1	2.00E-120	359
417	23803	UniRef90_H9J7G4 Uncharacterized protein n=2 Tax=Bombyx mori RepID=H9J7G4_BOMMO	65.28	47	72	0	6.00E-25	105
472	53334	UniRef90_H9JL73 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JL73_BOMMO	82.05	32	39	0	4.00E-11	72
472	53367	UniRef90_H9JL73 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JL73_BOMMO	82.05	32	39	0	4.00E-11	72
472	54957	UniRef90_G6DEB0 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6DEB0_DANPL	46.9	136	290	10	6.00E-40	157
475	48960	UniRef90_UPI0003501B9E PREDICTED: actin-binding protein anillin-like, partial n=1 Tax=Bombyx mori RepID=UPI0003501B9E	79.41	54	68	0	2.00E-16	84
475	48966	UniRef90_UPI0003501B9E PREDICTED: actin-binding protein anillin-like, partial n=1 Tax=Bombyx mori RepID=UPI0003501B9E	79.41	54	68	0	2.00E-16	84
475	49011	UniRef90_UPI0003501B9E PREDICTED: actin-binding protein anillin-like, partial n=1 Tax=Bombyx mori RepID=UPI0003501B9E	79.41	54	68	0	2.00E-16	84
487	6947	UniRef90_UPI000350050A PREDICTED: serine/threonine-protein kinase fused-like n=1 Tax=Bombyx mori RepID=UPI000350050A	85.07	57	67	1	3.00E-52	121
487	6964	UniRef90_UPI000350050A PREDICTED: serine/threonine-protein kinase fused-like n=1 Tax=Bombyx mori RepID=UPI000350050A	85.07	57	67	1	3.00E-52	121
514	10226	UniRef90_H9JN22 Uncharacterized protein n=1 Tax=Bombyx mori RepID=H9JN22_BOMMO	77.42	48	62	1	9.00E-21	101
534	50342	UniRef90_G6DBN7 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6DBN7_DANPL	79.63	43	54	0	8.00E-20	99.8
534	50395	UniRef90_G6DBN7 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6DBN7_DANPL	79.63	43	54	0	8.00E-20	99.8
534	50408	UniRef90_G6DBN7 Uncharacterized protein n=1 Tax=Danaus plexippus RepID=G6DBN7_DANPL	79.63	43	54	0	8.00E-20	99.8
538	3814	UniRef90_I4DL49 Microsomal glutathione S-transferase-like n=1 Tax=Papilio xuthus RepID=I4DL49_PAPXU	68.35	95	139	0	4.00E-64	210

Scaf is the spruce budworm reference scaffold, Pos is the position of the locus on the scaffolds, UniRef Hit is the top blastx hit UniRef90 protein, %id is the percentage of identical base matches between the locus and UniRef90 protein match, Id is the number of identical base matches, Len is the alignment length, Gap indicates the number of gap extensions in the alignment, evalue is the significance of the blastx hit, and Bit is the bit score of the hit.

Chapter 7

General Conclusions

Thesis Summary and Future Directions

The spruce budworm, *Choristoneura fumiferana,* species complex consists of some of North America's most significant defoliators of conifers (Volney and Fleming 2007; Nealis 2008). These species are irruptive pests with cyclical outbreak dynamics that cause serious economic losses to forestry (Maclean *et al*. 2001; Volney and Fleming 2007). In this thesis, I use a combination of traditional and novel genomic tools to characterize the extent of the genetic interactions between three species occurring in western Canada. These are the spruce budworm, *C. fumiferana*, the western spruce budworm, *C. occidentalis*, and the two-year-cycle spruce budworm, *C. biennis* whose species ranges meet along the Rocky Mountains of Alberta and British Columbia.

Development of tools for molecular ecology

The work presented in this thesis represents a methodological shift in the use of traditional tools for investigating population genetic variation to newer genome-wide approaches that harness the high-throughput capabilities of next-generation sequencing technologies. The use of mitochondrial cytochrome oxidase I (COI) DNA sequence and eight nuclear microsatellites allowed identification of individuals of both *C. occidentalis* and hybrid ancestry in a recent outbreak population in southwestern Alberta (Chapter 2), as well as several budworm species and hybrid types throughout other parts of Alberta and BC in previous work (Lumley and

Sperling 2011a, 2011b). However, these genetic markers were limited in their ability to distinguish between sympatric western species identified on the basis of life history characteristics. Lumley and Sperling (2011a) found that numerous western species, including *C. biennis*, *C. occidentalis*, *C. orae, C. lambertiana*, and *C. carnana*, formed a pair of western genetic clusters, despite having identifiable differences in pheromone attraction, host usage, and diapause characteristics. This inability to effectively distinguish between these species highlighted the need for additional genomic tools for investigation of the genetic relationships among these sympatric species.

A brief foray into microsatellite development from an existing genomic resource of expressed sequence tags (Chapter 3) showed some promise in providing additional genetic markers for these investigations. These loci have since been used to conduct an analysis of gene flow in a region called the Border Lakes Landscape in northern Minnesota and Ontario through collaborations with researchers from the United States Forest Service, Northern Forestry Centre and the Universities of Toronto and Montreal (James *et al*. in review). However, a technique called Genotyping-By-Sequencing (GBS) offered a more cost-efficient means of discovering and genotyping single nucleotide polymorphisms distributed throughout the genome. Using this approach I was able to use 2277 high-quality SNP loci to investigate hybridization and introgression between *C. fumiferana*, *C. biennis,* and *C. occidentalis* (Chapters 4 and 5). This change in methodology also marked an important shift from the use anonymous microsatellite markers, to physically mapped SNP loci via alignment with the spruce budworm reference genome. Furthermore, GBS allowed for the development of a preliminary spruce budworm genetic linkage map (Chapter 6).

Such accumulation of genomic resources for the spruce budworm species complex and other lepidopterans (Baxter *et al*. 2011; Rastas *et al.* 2013) can facilitate the transition of non-model organisms to model organism status for investigating evolutionary processes. This particularly applies to the characterization of the genetic basis of species boundaries between closely related species.

Spruce budworm hybridization

Hybridization within the spruce budworm species complex has been demonstrated extensively under controlled settings (Campbell 1958, 1967; Sanders *et al*. 1977; Harvey 1997). However, the results of Chapters 4 and 5 point to distinct species boundaries between the eastern, *C. fumiferana,* and western species, *C. biennis* and *C. occidentalis,* where these species meet in Alberta and BC. Populations were grouped into eastern and western genetic clusters across this region, with very few hybrids between these two groups. Only four individuals from three populations (WL, CL, and SR) east of the Rocky Mountains had admixed ancestries indicative of hybrids between eastern and western species (Figure 4-2). The situation between the two western species, however, was much different. Substructure within the western cluster was only weakly supported, resulting in subpopulations corresponding with *C. biennis* and *C. occidentalis* reference populations. These, however, had extensive allele sharing, and many more admixed individuals that were spatially distributed over many populations (Figure 4-4).

The contrasting patterns of admixture between these species point to differences in the maintenance of species boundaries between these species. Genomic and geographic clines analyses, in addition to the distribution of hybrid indices between eastern and western species,

show steep, concordant clines across most loci with significant allele frequency differences between them (Chapter 5). Similar patterns have been observed in other biological systems in which clear endogenous barriers to gene flow, such as the negative interaction of alleles that have diverged in isolation or Dobzhansky-Muller incompatibilities, are not known. Larson *et al*. (2013), for instance showed that the hybrid interactions between field crickets in eastern North America formed steep bimodal genotype distributions with few individuals of hybrid ancestry. However, the non-correspondence of these steep clines with exogenous factors led to Larson *et al*. to posit a role for non-ecological pre-mating barriers as the primary means of reproductive isolation between these species (Larson *et al*. 2013). On the other hand, the admixed individuals identified in this study between eastern and western spruce budworm species occur in a region where there is an equivalent shift from montane to boreal forest ecozones. This suggests that the rarity of hybrids between eastern and western spruce budworms may be a result of selection against hybrids maladapted to exogenous factors, such as habitat type. Nevertheless, pre-mating barriers appear to more developed within the complex, as in Larson *et al.* (2013), with differences in ecology (*e.g.* host use and phenology) and behavior (*e.g.* sex pheromone type and cross-attraction) being the primary barriers to gene flow.

It will be important to explore the capacity for postzygotic ecological inviability between eastern and western species, to render patterns consistent with the results presented in Chapters 4 and 5. This could be facilitated in at least two ways (Rundle and Whitlock 2001; Rundle and Nosil 2005): 1) comparison of hybrid fitness in natural and benign environments (Hatfield and Schluter 1999), such as on parental host trees and artificial diet; and 2) reciprocal transplantation of hybrid backcrosses in alternative parental environments (Rundle and Whitlock 2001; Rundle 2002). To my knowledge neither of these approaches has been attempted for spruce budworms.

Other likely alternative explanations for these patterns may reside in significant selection against immigrants as a result of assortative mating, or difficulties in mate-finding. However, it is not immediately clear how to approach testing these hypotheses in natural populations.

One particularly interesting result from this work was the finding of repeated patterns of asymmetry across multiple analyses. Analysis of several gene flow models between the three species suggest greater gene flow from west-to-east, as was predicted by Volney (1985) (Chapter 4). However, the 95% support limits for estimates of the effective number of migrants per generation in either direction were not mutually exclusive, and so the significance of this asymmetry is uncertain. Asymmetry was also evident in genomic and geographic clines (Chapter 5). The single most common homozygous genomic cline was one in which there were excesses of *C. occidentalis* alleles in *C. fumiferana* genetic backgrounds which would be consistent with west-to-east asymmetry (Table 5-2). However, over all homozygous genomic clines there were more loci with those showing excesses of *C. fumiferana* alleles in *C. occidentalis* genetic backgrounds, which is contradictory to west-to-east gene flow. Alternatively, geographic clines revealed greater variation in cline widths (and therefore introgression) in loci with clines centered further east (Figure 5-4), again supporting west-to-east gene flow.

While these patterns provide convincing evidence for asymmetry in the interactions between these species and west-to-east gene flow, it is important to distinguish between physical dispersal of individuals and the movement of genes. In fact, the populations on the receiving end of asymmetric introgression may be the party actively dispersing (*i.e.* invasive species, Currat *et al*. 2008). Thus, while introgression may be in the direction of west-to-east, this may result from greater westward dispersal of *C. fumiferana*. Thus, future work to characterize asymmetry in

gene flow between eastern and western spruce budworm species should focus on distinguishing between dispersal and introgression.

Systematic and taxonomic considerations

Finally, the work presented in this thesis highlights taxonomic challenges regarding the treatment of *C. biennis* and *C. occidentalis* as separate species. Genetic differentiation between western populations is very weak, and far below what is typically seen between species (Chapter 4). For instance, Powell *et al*. (2013) reported similar mean Fst values between host races of *Rhagoletis* flies. Similarly, despite using 2277 SNP loci distributed throughout the genome, population substructure correlating with reference populations of these species was weak and far less likely than that occurring between eastern and western species (Chapter 4). There were also no loci with fixed differences between species, in fact only eight loci had significant allele frequency differences greater than 0.5, and only two loci showed signs of significant genetic differentiation between reference population of these species.

Subtle differences in life history characteristics alone should not dictate the continued treatment of *C. biennis* and *C. occidentalis* as separate species, as these may result from ecologically-induced differential expression of phenotypic plasticity (Miner *et al.* 2005). The occurrence of second diapause (a defining species character of *C. biennis*), for instance, can also be induced in laboratory colonies of *C. occidentalis* (Nealis 2005). Thus, its occurrence in nature may instead be a result of parallel evolution of *C. occidentalis* populations locally adapted to cooler climates (Elmer and Meyer 2011; Butlin *et al.* 2014). Similar parallel divergences in reproductive timing have been described in another lepidopteran, *Inurois punctigera* (Yamamoto

et al. 2012). Thus, the lack of genomic integrity (Sperling 2003) in these two species and consistent ambiguity surrounding their phylogenetic relationships (Lumley and Sperling 2011a; Bird 2013) suggests that the treatment of *C. biennis* and *C. occidentalis* as distinct species requires revision. Accordingly, I suggest that *C. biennis* be sunk to subspecific status to reflect its weak geographic distinction from the core distribution of *C. occidentalis.*

Additional Future Directions

There are many research avenues that are currently in progress and others that remain to be investigated that either complement the work presented in this thesis or stem from it. Perhaps of most immediate interest is the desire to identify clines in non-molecular traits across the hybrid zone between eastern and western species, as phenotypic traits that differ between species are more likely to represent functional targets of selection (Linnen and Hoekstra 2010). Recently, Lumley and Sperling (2010) demonstrated through a quantitative analysis of wing colour and morphology that such traits were able to sufficiently distinguish between species. In addition, they presented evidence that *C. biennis* was intermediate in wing morphology characteristics to *C. fumiferana* and *C. occidentalis* (Lumley and Sperling 2010). Intermediacy of *C. biennis* is also apparent with respect to geography (Stehr 1967; Shepherd *et al.* 1995) and genitalic morphology (Gray *et al*. 1995), suggesting that its biology may be heavily influenced by introgression from *C. fumiferana* and *C. occidentalis*. Thus, it will be interesting to determine if equivalent clines across the hybrid zone between eastern and western species are also apparent in wing colour traits and genitalic characteristics.

A topic that is of special interest to me concerns the role of the sex chromosome in spruce budworm evolution. A study conducted by Sperling (1994) demonstrated a peculiar pattern in linkage relationships of species differences within the spruce budworm species complex. Sperling (1994) noted that nearly all species differences that have been characterized between spruce budworms have at least partial linkage to the sex chromosome. The underlying genetic basis for this remarkable bias in the distribution of these differences, however, has remained unanswered. I hope that, through continued development of the linkage map presented in Chapter 6 and by mapping quantitative trait loci, the genetic architecture of spruce budworm species differences will eventually be revealed. In addition, more in-depth genome scan approaches that examine the extent of spatial autocorrelation and heterogeneity of highly differentiated regions along chromosome lengths may reveal important insights into the role of genome structure in local adaptation (Renaut *et al.* 2012; Andrews *et al*. 2013; Powell *et al.* 2013).

Lastly, the factors controlling synchronicity in budworm outbreak cycles across vast landscapes remain largely unknown. Bioclimatic factors (Candau and Fleming 2005) and host connectivity (Robert *et al.* 2012), among other factors, are both presumed to play an integral role in facilitating or limiting dispersal between budworm populations. Thus, any effort to illuminate the relationships between these factors and underlying spruce budworm genetic variation will greatly improve predictive dispersal models and aid in better management of forest resources. As such, research is underway to characterize the integrated landscape genetics of spruce budworms across the province of Alberta.

In conclusion, although the spruce budworm species complex is rife with intricacies, it is this complexity that has fascinated scientists for generations. The work presented in this thesis

contributes to a voluminous body of research on this significant defoliator of North American conifers, and furthers our understanding of the evolutionary relationships between these species. With time, patience, and curiosity, the mysteries surrounding the origins, biology, and dynamics of this peculiar group of moths will continue to unfold.

Literature Cited

Andrews, R.L., and L.H. Rieseberg. 2013. Divergence is focused on few genomic regions early in speciation: incipient speciation of sunflower ecotypes. Evolution, 67-9: 2468-2482.

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., and M.L. Blaxter. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. PLoS One, 6(4): e19315. doi:10.1371/journal.pone.0019315

Bird, H.M. 2013. Phylogenomics of the Choristoneura fumiferana species complex (Lepidopter: Tortricidae). MSc Thesis, University of Alberta, Edmonton, Canada.

Butlin, R.K., Saura, M., Charrier, G., Jackson, B., Andre, C., Cabellero, A., Coyne, J.A., Galindo, J., Grahame, J.W., Hollander, J., Kemppainen, P., Martinez-Fernandez, M., Panova, M., Quesada, H., Johannesson, K., and E. Rolan-Alvarez. 2014. Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. Evolution, 68: 935-949.

Campbell, I.M. 1958. A genetic study of factors determining fecundity in the genus *Choristoneura* Lederer (Clem.) (Tortricidae). PhD Thesis, University of Toronto, Toronto, Canada.

Campbell, I.M. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist, 99: 482-486.

Candau, J.-N., and R.A. Fleming. 2005. Landscape-scale spatial distribution of spruce budworm defoliation in relation to bioclimatic conditions. Canadian Jounral of Forest Research, 35: 2218-2232.

Currat, M., Ruedi, M., Petit, R.J., and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution, 62: 1908-1920.

Elmer, K.R., and A. Meyer. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends in Ecology and Evolution, 26: 298-306.

Gray, T.G., Shepherd, R.F., and G.T. Harvey. 1995. Incidence of spicules on the aedeagi of *Choristoneura fumiferana, C. biennis,* and *C.*unidentified species (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 161-166.

Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferphagous *Choristoneura* (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519-536.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. Evolution, 53: 866-873.

Larson, E.L., White, T.A., Ross, C.L., and R.G. Harrison. 2013. Gene flow and the maintenance of species boundaries. Molecular Ecology. Doi:10.111/mec.12601

Linnen, C.R., and H.E. Hoekstra. 2010. Measuring natural selection on genotypes and phenotypes in the wild. Cold Spring Harbor Symposia on Quantitative Biology, 74: 155-168.

Lumley, L.M., and F.A.H. Sperling. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology, 35: 416-428.

Lumley, L.M., and F.A.H. Sperling. 2011a. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232-243.

Lumley, L.M., and F.A.H. Sperling. 2011b. Life-history traits maintin the genomic integrity of sympatric species of the spruce budworm (*Choristoneura fumiferana*) group on an isolated forest island). Ecology and Evolution, 1: 119-131.

MacLean, D.A., Erdle, T.A., MacKinnon, W.E., Porter, K.B., Beaton, K.P., Cormier, G., Morehouse, S., and M. Budd. 2001. The spruce budworm decision support system: forest protection planning to sustain long-term wood supply. Canadian Journal of Forest Research, 31: 1742-1757.

Miner, B.G, Sultan, S., Morgan, S.G., Padila, D.K., and R.A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution, 20: 685-692.

Nealis, V.G. 2005. Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist 137: 584-597.

Nealis, V.G. 2008. Spruce budworms, *Choristoneura* Lederer (Lepidoptera: Tortricidae). Pp. 3524-3531 in J.L. Capinera (Ed.), Encyclopedia of Entomology, 2nd edition. Springer, Dordrecht. Pp. 4411.

Powell, T.H.Q., Hood, G.R., Murphy, M.O., Heilveil, J.S., Berlocher, S.H., Nosil, P., and J.L., Feder. 2013. Genetic divergence along the speciation continuum: the transition from host race to species in Rhagoletis (Diptera: Tephritidae). Evolution, 67: 2561-2576.

Rastas, P., Paulin, L., Hanski, I., Lehtonen, R., and P. Auvinen. 2013. Lep-MAP: fast and accurate linkage map construction for large SNP datasets. Bioinformatics, 29: 3128-3134.

Renaut, S., Maillet, N., Normandeau, E., Sauvage, C., Derome, N., Rogers, S.M., and L. Bernatchez. 2012. Genome-wide patterns of divergence during speciation: the lake whitefish case study. Philosophical transactions of the Royal Society B, 367: 354-363.

Robert, L.-E., Kneeshaw, D., and B.R. Sturtevante. 2012. Effects of forest management legacies on spruce budworm (*Choristoneura fumiferana*) outbreaks. Canadian Jounral of Forest Research, 42: 463-475.

Rundle, H.D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. Evolution, 56: 322-329.

Rundle, H.D., and P. Nosil. 2005. Ecological speciation. Ecology letters, 8: 336-352.

Rundle, H.D., and M.C. Whitlock. 2001. A genetic interpretation of ecologically dependent isolation. Evolution, 55: 198-201.

Shepherd, R.F., Gray, T.G., and G.T. Harvey. 1995. Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies, Picea,* and *Pseudotsuga* in western Canada and Alaska. The Canadian Entomologist, 127: 813-830.

Sanders, C.J., Daterman, G.E., and T.J. Ennis. 1977. Sex pheromone responses of *Choristoneura* spp. and their hybrids (Lepidoptera: Tortricidae). The Canadian Entomologist, 109: 1203-1220.

Sperling, F.A.H. 1994. Sex-linked genes and species differences in Lepidoptera. The Canadian Entomologist, 126: 807-818.

Sperling, F.A.H. 2003. Butterfly Molecular Systematics: from species defintions to higher-level phylogenies. Pp. 431-458 in C.L. Boggs, W.B. Watt, and P.R. Ehrlich (Eds.), Butterflies: Ecology and Evolution taking flight. University of Chicago Press.

Stehr, G.W. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America. II. Geographic distribution in accordance with forest regions. The Canadian Entomologist, 99: 456-463.

Volney, W.J.A. 1985. Comparative population biologies of North American spruce budworms. Pp. 71-84 in C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Volney, W.J.A., and R.A. Fleming. 2007. Spruce budworm (*Choristoneura* spp.) biotype reactions to forest and climate characterisitics. Global Change Biology, 13: 1630-1643.

Yamamoto, S., and T. Sota. 2012. Parallel allochronic divergence in a winter moth due to disruption of reproductive period by winter harshness. Molecular Ecology, 21: 174-183.

Biography

I was born on August 2, 1983 at the Civic Hospital in Ottawa, Ontario as the first of three children to my parents, Thomas Brunet and Debbie Zieriesen (née Halan, formerly Brunet). I have many fond memories from my childhood, including chasing raccoons up telephone poles in my backyard (all the while believing that they were rabbits), family excursions to the Central Experimental Farms where I would develop my entomological interests later on in life, exploring the Mississippi River in the town of Carleton Place, saving "bugs" from my backyard pool, and camping trips with my extended family. My childhood was a very active one as I played baseball and took up both Judo and Karate before finally settling into competitive swimming.

Following high school, I knew I wanted to continue my education in a biological field and enrolled in a general biology undergraduate program at the University of Ottawa. During this time I participated in a co-operative work placement-education program, where I obtained my first real entomological experience. I was employed by Dr. Robert Foottit at Agriculture and Agri-food Canada's Eastern Cereal and Oilseed Research Centre in the summer of 2004 as an assistant curator to the aphid unit of the Canadian National Collection (CNC) of Insects, Arachnids, and Nematodes. I spent much of the next four years working in this facility under the tutelage of Dr. Foottit and his technician Eric Maw, as well as Dr. Gary Gibson, completing all of my undergraduate work placement requirements, an eight month honours project on aphid morphometrics, and contractual work to inventory the CNC's Chalcidoidea collections and identify aphids from a long-term project on plum-pox virus transmission in southern Ontario.

I continued to swim competitively, donning the University of Ottawa's garnet and grey varsity colours for four years. I also worked as a lifeguard at several pools and beaches in and

around Ottawa. In 2004, whilst preparing for a much anticipated national lifeguarding competition, I met Staci Forsen and the two of us have been inseparable ever since.

Upon completion of my undergraduate degree in 2007, Staci and I ventured west to Edmonton to partake in post-degree programs at the University of Alberta. In 2008, I started my MSc in Dr. Felix Sperling's lab, switching into a PhD in 2010. My time here has been an incredible experience, allowing me to gain valuable field and lab skills, as well as introducing me to a great group of people with whom I share many interests. It has also strengthened my relationship with Staci; on the 27th of July, 2012 we were married, and our son Luc was delivered on the 8th of June, 2014.

Bibliography

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Most, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice., A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., stelkens, R., Szymura, J.M., Vainola, R., Wolf, J.B.W., and D. Zinner. 2012. Hybridization and speciation. Journal of Evolutionary Biology, 26: 229-246.

Alberta Environment and Sustainable Resource Development. 2005. Forest Health in Alberta, 2005 Annual Report. Edmonton, Alberta. Pp. 22.

Alberta Environment and Sustainable Resource Development. 2007. Forest Health in Alberta, 2006 Annual Report. Edmonton, Alberta, Pp. 58.

Alberta Environment and Sustainable Resource Development. 2009. Forest Health in Alberta, 2008 Annual Report. Edmonton, Alberta, Pp. 50.

Alberta Environment and Sustainable Resource Development. 2010. Forest Health in Alberta, 2009 Annual Report. Edmonton, Alberta, Pp. 48.

Alberta Environment and Sustainable Resource Development. 2011. Forest Health in Alberta, 2010 Annual Report. Edmonton, Alberta, Pp. 54.

Anderson, D.P., and B.R. Sturtevant. 2011. Pattern analysis of eastern spruce budworm Choristoneura fumiferana dispersal. Ecography, 34: 488-497.

Anderson, E. 1948. Hybridization of the habitat. Evolution, 2: 1-9.

Anderson, E.C., and E.A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics, 160: 1217-1229.

Andolfatto, P. Davison, D., Erezyilmaz, D., Hu, T.T., Mast, J., Sunayama-Moritaa, T., and D. Stern. 2011. Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome Research, 21: 610-617.

Andrew, R.L., and L.H. Rieseberg. 2013. Divergence is focused on few genomic regions early in speciation: incipient speciation of sunflower ecotypes. Evolution, 67-9: 2468-2482.

Arnold, M.L., and N.H. Martin. 2009. Adaptation by introgression. Journal of Biology, 8: 82.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., and E.A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One, 3(10);e3376. Doi:10.1371/journal.pone.0003376

Barton, N., and K. Gale. 1993. Genetic analysis of hybrid zones. In: R.G. Harrison (ed.) Hybrid zones and the evolutionary process. Oxford University Press, Oxford. Pp 13-45.

Barton, N.H., and G.M. Hewitt. 1985 Analysis of hybrid zones. Annual Review of Ecology and Systematics, 16: 113-148.

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., and M.L. Blaxter. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. PLoS One, 6(4): e19315. doi:10.1371/journal.pone.0019315

Beaumont, M.A., and D.J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology, 13: 969-980.

Beaumont, M.A., and R.A. Nichols. 1996. A method for quantifying differentiation between populations at multi-allelic loci and its implications for investigating identity and paternity. Genetica, 96: 3-12.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics, 22: 341-345.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy Sciences of the United States of America, 98: 4563-4568.

Beerli, P., M. Palczewski. 2010. Unified framwork to evaluate panmixa and migration direction among mulitple sampling locations. Genetics, 185: 313-326.

Beldade, P., Saenko, S.V., Pul, N., and A.D. Long. 2009. A gene-based linkage map for Bicyclus anynana butterflies allows for a comprehensive analysis of synteny with the lepidopteran reference genome. PLoS Genet 5(2): e1000366. Doi:10.1371/journal.pgen.1000366.

Beldade, P., van't Hoff, A., Jeronimo, M. A., and A.D. Long. 2009. Microsatellite markers associated with genes expressed in developing wings of Bicyclus anynana butterflies. Molecular Ecology Resources, 9: 1487-1492.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57: 289-300.

Beysard, M. and G. Heckel. 2014. Structure and dynamics of hybrid zones at different stages of speciation in the common vole (Microtus arvalis). Molecular Ecology, 23: 673-687.

Bierne, N., Welch, J., Loire, E., Bonhomme, F., and P. David. 2011. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Molecular Ecology, 20: 2044-2072.

Bird, H.M. 2013. Phylogenomics of the Choristoneura fumiferana species complex (Lepidopter: Tortricidae). MSc Thesis, University of Alberta, Edmonton, Canada.

Bradshaw, Jr. H.D., Otto, K.G., Frewen, B.E., McKay, J.K., and D.W., Schemske. 1998. Quantitative trait loci affecting difference in floral morphology between two species of monkeyflower (Mimulus). Genetics, 149: 367-382.

Bridle, J.R., and T.H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends in Ecology and Evolution, 22: 140-147.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences, 91: 6491-6495.

Brunet, B.T.M., Doucet, D., Sturtevant, B.R., and F.A.H. Sperling. 2013. Characterization of EST-based SSR loci in the spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae). Conservation Genetic Resources, 5: 541-544.

Brunet, B.T.M., Hundsdoerfer, A.E., and F.A.H. Sperling. 2013. Identification and ecological characterisation of Choristoneura occidentalis (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada. The Canadian Entomologist, 145: 521-528.

Buerkle, C.A. 2005. Maximum-likelihood estimation of a hybrid index based on molecular markers. Molecular Ecology Notes, 5: 684-687.

Buerkle, C.A., and L.H. Rieseberg. 2001. Low intraspecific variation for genomic isolation between hybridizing sunflower species. Evolution, 55: 684-691.

Buerkle, C.A., Morris, R.J., Asmussens, M.A., and L.H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. Heredity, 84: 441-451.

Butlin, R. 1987 Speciation by reinforcement. Trends in Ecology and Evolution, 2: 8-13.

Butlin, R.K., Saura, M., Charrier, G., Jackson, B., Andre, C., Cabellero, A., Coyne, J.A., Galindo, J., Grahame, J.W., Hollander, J., Kemppainen, P., Martinez-Fernandez, M., Panova, M., Quesada, H., Johannesson, K., and E. Rolan-Alvarez. 2014. Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. Evolution, 68: 935-949.

Cadogan, B.L., Nealis, V.G., and K. van Frankenhuyzen. 1995. Control of spruce budworm (Lepidoptera: Tortricidae) with *Bacillus thuringiensis* applications times to conserve a larval parasitoid. Crop protection, 14: 31-36.

Campbell, I.M. 1958. A genetic study of factors determining fecundity in the genus Choristoneura Lederer (Clem.) (Tortricidae). PhD Thesis, University of Toronto, Toronto, Canada.

Campbell, I.M. 1962. Reproductive capacity in the genus Choristoneura Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers or rates. Canadian Journal of Genetics and Cytology, 4: 272-288.

Campbell, I.M. 1967. On coniferophagous species of Choristoneura (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist 99: 482-486.

Campbell, I.M. 1967. On coniferophagous species of Choristoneura (Lepidoptera: Tortricidae) in North America. IV. Sexual isolation between three species. The Canadian Entomologist, 99: 482-486.

Candau, J.-N., and R.A. Fleming. 2005. Landscape-scale spatial distribution of spruce budworm defoliation in relation to bioclimatic conditions. Canadian Jounral of Forest Research, 35: 2218-2232.

Carling, M.D., and R.T. Brumfield. 2009. Speciation in passerina buntings: introgression patterns of sex-linked loci identifying a candidate gene region for reproductive isolation. Molecular Ecology, 18: 834-847.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., and J.H., Postlethwait. 2011. Stacks: Building and genotyping loci De novo from short-read sequences. Genes Genomes Genetics, 1: 171-182. Doi:10.1534/g3.111.000240.

Catchen, J.M., Hohenlohe, P.A., Bassham, S., Amores, A., and W.A., Cresko. 2013, Stacks: an analysis tool set for population genomics. Molecular Ecology, 22: 3124-3140.

Chenuil, A. 2012. How to infer reliable diploid genotypes from NGS or traditional sequence data: from basic probability to experimental optimization. Journal of Evolutionary Biology, 25: 949-960.

Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., and M. Robles. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21: 3674-3676.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinuaer Associates, Inc., Sunderland, Massachusetts. Pp. 543.

Cullingham, C.I., Cooke, J.E.K., Dang, S., Davis, C.S., Cooke, B.J., and D.W. Coltman. 2011. Mountain pine beetle host-range expansion threatens the boreal forest. Molecular Ecology, 20: 2157–2171.

Currat, M., Ruedi, M., Petit, R.J., and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution, 62: 1908-1920.

Danecek, P., Auton, A., Abecasis, G., Albers, C., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics, 27: 2156-2158.

Dang, P.T. 1985. Key to adult males of conifer-feeding species of Choristoneura (Lepidoptera: Tortricidae) in Canada and Alaska. The Canadian Entomologist, 117: 1-5.

Dasmahapatra, K.K., Walters, J.R., Briscoe, A.D., Davey, J.W., Whibley, A., Nadeau, N.J., Zimin, A.V., Hughes, D.S.T., Ferguson, L.C., Martin, S.H., Salazar, C., Lewis, J.J., Adler, S., Ahn, S.-J., Baker, D.A., Baxter, S.W., Chamberlain, N.L., Chauhan, R., Counterman, B.A., Dalmay, T., Gilbert, L.E., Gordon, K., Heckel, D.G., Hines, H.M., Hoff, K.J., Holland, P.W.H., Jacquin-Joly, E., Jiggins, F.M., Jones, R.T., Kapan, D.D.,

Kersey, P., Lamas, G., Lawson, D., Mapleson, D., Maroja, L.S., Martin, A., Moxon, S., Palmer, W.J., Papa, R., Papanicolaou, A., Pauchet, Y., Ray, D.A., Rosser, N., Salzberg, S.L., Supple, M.A., Surridge, A., Tenger-Trolander, A., Vogel, H., Wilkinson, P.A., Wilson, D., Yorke, J.A., Yuan, F., Balmuth, A.L., Eland, C., Gharbi, K., Thomson, M., Gibbs, R.A., Han, Y., Jayaseelan, J.C., Kovar, C., Mathew, T., Muzny, D.M., Ongeri, F., Pu, L.-L., Qu, J., Thornton, R.L., Worley, K.C., Wu, Y.-Q., Linares, M., Blaxter, M.L., ffrench Constant, R.H., Joron, M., Kronforst, M.R., Mullen, S.P., Reed, R.D., Scherer, S.E., Richards, S., Mallet, J., McMillan, W.O., and C.D. Jiggins. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature, 487: 94-98.

Daterman, G.E., Sower, L.L., Stevens, R.E., and D.G. Fellin. 1995. Pheromone chemistry and response behaviour of conifer-feeding Choristoneura in the western United States. Pp. 69-84 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding Choristoneura (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Davey, J.W., and M.L. Blaxter. 2010. RADSeq: next-generation population genetics. Briefings in Functional Genomics, 9: 416-423.

Davey, J.W., Cezard, T., Fuentes-Utrilla, P., Eland, C., Gharbi, K., and M.L. Blaxter. 2013. Special features of RAD sequening data: implications for genotyping. Molecular Ecology, 22: 3151-3164.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., and M.L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12: 499-510.

de Arruda, M.P., Goncalves, E.C., Schneider, M.P.C., da Costa da Silva, A.L., and E. Morielle-Versute. 2010. An alternative genotyping method using dye labeled universal primer to reduce unspecific amplifications. Molecular Biology Reports, 37: 2031-2036.

De Benedictis, J.A., Liebhold, A.M., and J.A. Powell. 1995. Studies of pheromone attraction and inheritance using virgin females of Choristoneura species, in the Central Sierra Nevada, California. Pp. 151-166 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding Choristoneura (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Deverno, L.L., Smith,. G.A., and K.J. Harrison. 1998. Randomly amplified polymorphic DNA evidence of introgression in two closely related sympatric species of coniferophagous Choristoneura (Lepidoptera: Tortricidae) in Atlantic Canada. Annals of the Entomological Society of America, 91: 248-259.

Dobesberger, E.J., Lim, K.P., and A.G. Raske. 1983. Spruce budworm (Lepidoptera: Tortricidae) moth flight from New Brunswick to Newfoundland. The Canadian Entomologist, 115: 1641-1645.

Earl, D.A., and B.M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetic Resources, 4: 359-361.

Ekblom, R., and J. Galindo. 2010. Applications of next generation sequencing in molecular ecology of non-model organsims. Heredity, 107: 1-15.

Elmer, K.R., and A. Meyer. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends in Ecology and Evolution, 26: 298-306.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and S.E., Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One, 6(5): e19379. doi:10.10371/journal.pone.0019379.

Ennis, T.J. 1976. Sex chromatin and chromosome numbers in Lepidoptera. Canadian Journal of Genetics and Cytology, 18: 119-130.

Evanno, G., Regnaut, S., and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14: 2611-2620.

Ewing, B., Hillier, L., Wendl, M., and P. Green. 2008. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Research, 8: 175-185.

Excoffier, L., Hofer, T., and M. Foll. 2009. Detecting loci under selection in a hierarchically structured population. Heredity, 103: 285-298.

Falush, D., Stephens, M., and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164: 1567-1587.

Farrar, J.L. 1995. Trees in Canada. Natural Resources Canada, Canadian Forest Service, Ottawa, Ontario, Canada and Fitzhenry and Whiteside Limited, Markham, Ontario, Canada.

Fellin, D.G., and J.E. Dewey. 1982. Western spruce budworm. Forest Insect & Disease Leaflet 53, United States Department of Agriculture, Forest Service, Missoula, Montana, United States of America.

Fitzpatrick, B. 2013. Alternative forms for genomic clines. Ecology and Evolution, doi: 10.1002/ece3.609.

Fitzpatrick, B.M. 2012. Estimating ancestry and heterozygosity of hybrids using molecular markers. BMC Evolutionary Biology, 12: 131. https://www.biomedcentral.com/1471-2148/12/131

Floate, K.D., and T.G. Whitham. 1993. The "Hybrid Bridge" hypothesis: host shifting via plant hybrid swarms. The American Naturalist, 141: 651–662.

Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics, 180: 977-993.

Fraser, D.J., Lippe, C., and L. Bernatchez. 2003. Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Molecular Ecology, 13: 67-80.

Freeman, T.N. 1947. A new generic assignment for Archips fumiferana (Clemens), the spruce budworm (Lepidoptera, Tortricidae). The Canadian Entomologist, 79: 21-31.

Freeman, T.N. 1967. On coniferophagous species of Choristoneura (Lepidoptera: Tortricidae) in North America. I. Some new forms of Choristoneura allied to C. fumiferana. The Canadian Entomologist, 99: 449-455.

Gadau, J., Page, Jr. R.E., J.H. Werren. 2002. Mapping of hybrid incompatibility loci in Nasonia. Genetics, 153: 1731-1741.

Gagnaire, P.-A., Pavey, S.A., Normandeau, E., and L. Bernatchez. 2013. The genetic architecture of reproductive isolation during speciation-with-gene-flow in lake whitefish species pairs assessed by RAD sequencing. Evolution, 67: 2483-2497.

Gardner, M.G., Fitch, A.J., Bertozzi, T., and A.J. Lowe. 2011. Rise of the machines - recommendations for ecologists when using next generation sequencing for microsatellite development. Molecular Ecology Resources, 11: 1093-1101.

Gilligan, T.M., and J.W. Brown. 2014. A new name for the western spruce budworm (Lepidoptera: Tortricidae)? The Canadian Entomologist, 00: 1-7.

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q., and E.S. Buckler. 2014. TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS ONE 9(2): e90346. Doi:10.1371/journal.pone.0090346.

Gompert, Z., and C.A. Buerkle. 2009. A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. Molecular Ecology, 18: 1207-1224.

Gompert, Z., and C.A. Buerkle. 2010. INTROGRESS: a software package for mapping components of isolation in hybrids. Molecular Ecology Resources, 10: 378-384.

Gompert, Z., and C.A. Buerkle. 2011. Bayesian estimation of genomic clines. Molecular Ecology, 20: 2111-2127.

Gray, T.G., Shepherd, R.F., and G.T. Harvey. 1995. Incidence of spicules on the aedeagi of Choristoneura fumiferana, C. biennis, and C.unidentified species (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 161-166.

Greenbank, D.O., Schaefer, G.W., and R.C. Rainey. 1980. Spruce budworm (Lepidoptera: Tortricidae) moth flight and dispersal: new understanding from canopy observations, radar, and aircraft. Memoirs of the Entomological Society of Canada, No. 110. pp. 49.

Groot, A.T., Staudacher, H., Barthel, A., Inglis, O., Schofl, G., Santangelo, R.G., Gebauer-jung, S., Vogel, H., Emerson, J., Schal, C., Heckel, D.G., and F. Gould. 2013. One quantitative trait locus for intra- and interspecific variation in a sex pheromone. Molecular Ecology, 22: 1065-1080.

Hamilton, J.A., Lexer, C., and S.N. Aitken. 2012. Differential introgression reveals candidate genes for selection across a spruce (Picea sitchensis x P. glauca) hybrid zone. New Phytologist, doi: 10.1111/nph.12055.

Hamilton, J.A., Lexer, C., and S.N. Aitken. 2012. Genomic and phenotypic architecture of a spruce hybrid zone (Picea sitchensis x P. glauca). Molecular Ecology, doi:10.1111/mec.12007.

Harrell, F.E. Jr. with contributions from Charles Dupont and many others. 2013. Hmisc: Harrell Miscellaneous. R package version 3.12-2. http://CRAN.R-project.org/package=Hmisc.

Harvey, G.T. 1957. The occurrence and nature of diapause-free development in the spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricidae). The Canadian Jounral of Zoology, 35: 549-572.

Harvey, G.T. 1961. Second Diapause in spruce budworm from eastern Canada. The Canadian Entomologist, 93: 594-602.

Harvey, G.T. 1967. On coniferophagous species of Choristoneura (Lepidoptera: Tortricidae) in North America V. Second diapause as a species character. The Canadian Entomologist, 99: 486-503.

Harvey, G.T. 1977. Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 109: 487-496.

Harvey, G.T. 1983a. A geographical cline in egg weights in Choristoneura fumiferana (Lepidoptera: Tortricidae) and its significance in population dynamics. The Canadian Entomologist, 115: 1103-1108.

Harvey, G.T. 1983b. Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 115: 1109-1117.

Harvey, G.T. 1985a. The taxonomy of coniferophagous Choristoneura (Lepidoptera: Tortricidae): A review. Pp. 16-59 in C.J. Sanders, R.W. Stark, E.J. Mullins, And J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Harvey, G.T. 1985b. Egg weight as a factor in the overwintering survival of spruce budworm (Lepidoptera: Tortricidae) larvae. The Canadian Entomologist, 117: 1451-1461.

Harvey, G.T. 1997. Interspecific crosses and fertile hybrids among the coniferophagous Choristoneura (Lepidoptera: Tortricidae). The Canadian Entomologist, 129: 519–536.

Haselhorst, M.S.H., and C.A. Buerkle. 2013. Population genetic structure of Picea engelmannii, P. glauca and their previously unrecognized hybrids in the central Rocky Mountains. Tree Genetic & Genomes, 9: 669-681.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. Evolution, 53: 866-873.

Heckel, D.G., Gahan, L.J., Liu, Y.-B., and B.E. Tabashnik. 1999. Genetic mapping of resistance to Bacillus thuringiensis toxins in diamondback moth using biphasic linkage analysis. Proceedings of the National Academy of Sciences of the United States of America, 96: 8373-8377.

Hedrick, P.W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology, 22: 4606-4618.

Hewitt, G.M. 1988. Hybrid zones - Natural Laboratories for evolutionary studies. Trends in Ecology and Evolution, 3: 158-167.

Hijmans, R.J., Williams, E.D., and C. Vennes. 2012. geosphere: Spherical trigonometry. R package version 1.2-28. http://CRAN.R-project.org/package=geosphere.

Hohenlohe, P.A., Amish, S.J., Catchen, J.M., Allendorf, F.W., and G. Luikart. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources, 11: 117-122.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A., and W.A. Cresko. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genetics, 6:e1000862. Doi:10.1371/journal.pgen.1000862.

Holt, R.D., Knight, T.M., and M. Barfield. 2004. Allee effects, immigration, and the evolution of species' niches. The American Naturalist, 163: 253-262.

Jakobsson, M. and N.A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23: 1801-1806.

Jiggins, C. D., Mavarez, J., Beltran, M., McMillan, W.O., Spencer, J.S., and E. Bermingham. 2005. A genetic linkage map of the mimetic Butterfly Heliconius melpomene. Genetics, 171: 557-570.

Jiggins, C.D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in Ecology and Evolution, 15: 250-255.

Jin, X., Ho, T.S., and D.P. Smith. 2008. SNMP is a signaling component required for pheromone sensitivity in Drosophila. Proceedings of the National Academy of Sciences, 105: 10996-11001.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structure populations. BMC Genetics, 11: 94. http://www.biomedcentral.com/1471-2156/11/94

Kapan, D.D., Flanagan, N.S., Tobler, A., Papa, R., Reed, R.D., Acevedo Gonzalez, J., Ramirez Restreppo, M., Martinez, L., Maldonato, K., Ritschoff, C., Heckel, D.G., and W.O. McMillan. 2007. Localization of Mullerian mimicry genes on a dense linkage map of Heliconius erato. Genetics, 173: 735-757.

Kim, K. S., Ratcliffe, S. T., French, B. W., Liu, L., and T.W. Sappington. 2008. Utility of EST-derived SSRs as population genetic markers in a beetle. Journal of Heredity, 99: 112-124.

Languth, E.L., Fedy, B.C., Oyler-McCance, S.J., Garey, A.L., Emel, S.L., Mumma, M., Wagner, H.H., Fortin, M.-J., and S.A. Cushman. 2011. Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. Molecular Ecology Resources, 12: 276-284.

Larson, E.L., White, T.A., Ross, C.L., and R.G. Harrison. 2013. Gene flow and the maintenance of species boundaries. Molecular Ecology. Doi:10.111/mec.12601

Latch, E.K., Heffelfinger, J.R., Fike, J., and O.E., Rhodes. 2009. Species-wide phylogeography of North American mule deer (Odocoileus hemionus): cryptic glacial refugia postglacial recolonization. Molecular Ecology, 18: 1730-1745.

Lawniczak, M.K.N., Emrich, S.J., Holloway, A.K., Regier, A.P., Olson, M., White, B., Redmond, S., Fulton, L., Appelbaum, E., Godfrey, J., Farmer, C., Chinwalla, A., Yang, S.-P., Minx, P., Nelson, J., Kyung, K., Walenz, B.P., Garcia-Hernandez, E., Aguiar, M., Viswanathan, L.D., Rogers, Y.-H., Strausberg, R.L., Saski, C.A., Lawson, D., Colins, F.H., Kafatos, F.C., Christophides, G.K., Clifton, S.W., Kirkness, E.F., and N.J., Besansky. 2010. Widespread divergence between incipient Anopheles gambiae species revealed by whole genome sequences. Science, 330: 512-514.

Lawrence, R.K., Mattson, W.J., and R.A., Haack. 1997. White spruce and the spruce budworm: defining the phonological window of susceptibility. The Canadian Entomologist, 129: 291-318.

Le Corre, V., and A. Kremer. 2012. The genetic diiferentiation at quantitative trait loci under local adaptation. Molecular Ecology, 21: 1548-1566.

Lewontin, R.C. and J. Krakauer. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics, 74: 175-195.

Lexer, C., Kremer, A., and R.J. Petit. 2006. Shared alleles in sympatric oaks: recurrent gene flow is a more parsimonius explanation than ancestral polymorphism. Molecular Ecology, 15: 2007-2012.

Lexer, C., Rosenthal, D.M., Raymond, O., Donovan, L.A., and L.H., Rieseberg. 2005. Genetics of species differences in the wild annual sunflowers Helianthus annuus and H. petiolaris. Genetics, 169: 2225-2239.

Leyva, K.J., Clancy, K.M., and P.W. Price. 2000. Oviposition preference and larval performance of the western spruce budworm (Lepidoptera: Tortricidae). Environmental Entomology, 29: 281–289.

Leyva, K.J., Clancy, K.M., and P.W. Price. 2003. Oviposition strategies employed by the western spruce budworm: tests of predictions from the phylogenetic constraints hypothesis. Agricultural and Forest Entomology, 5: 9–16.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-1760.

Liebhold, A.M. 1986. Larval coloration of hybrids between Choristoneura occidentalis and C. retiniana (Lepidoptera: Tortricidae). The Canadian Entomologist, 118: 857-860.

Liebhold, A.M., and W.J.A. Volney. 1984. Effect of temporal factors on reproductive isolation between Choristoneura occidentalis and C. retiniana (Lepidoptera: Tortricidae). The Canadian Entomologist, 116: 991-1005.

Liebhold, A.M., Volney, W.J.A., and W.E. Waters. 1984. Evaluation of cross-attraction between sympatric Choristoneura occidentalis and C. retiniana (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 827-840.

Linnen, C.R., and H.E. Hoekstra. 2010. Measuring natural selection on genotypes and phenotypes in the wild. Cold Spring Harbor Symposia on Quantitative Biology, 74: 155-168.

Logan, J.A., Bentz, B.J., Vandygriff, J.C., and D.L. Turner. 1998. General program for determining instar distributions from widths: example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. Environmental Entomology, 27: 555–563.

Lotterhos, K.E., and M.C. Whitlock. 2014. Evaluation of demographic history and neutral parameterization on the performance of Fst outlier tests. Molecular Ecology, doi: 10.1111/mec/12725.

Lukhtanov, V.A. 2000. Sex chromatin and sex chromosome systems in nonditrysian Lepidoptera (Insecta). Journal of Zoological Systematics and Evolutionary Research, 38: 73-79.

Lumley, L. M., Davis, C. S., and F.A.H. Sperling. 2009. Isolation and characterization of eight microsatellite loci in the spruce budworm species Choristoneura fumiferana and

Choristoneura occidentalis, and cross-species amplification in related tortricid moths. Conservation Genetics Resources, 1: 501-504.

Lumley, L.M. 2010. Species delimitation in the *Choristoneura fumiferana* species complex (Lepidoptera: Tortricidae). Ph. D. Thesis. University of Alberta, Edmonton, Canada.

Lumley, L.M., and F.A.H. Sperling. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (Choristoneura fumiferana) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology, 35: 416-428.

Lumley, L.M., and F.A.H. Sperling. 2011a. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (Choristoneura fumiferana) species complex (Lepidoptera: Tortricidae). Molecular Phylogenetics and Evolution, 58: 232-243.

Lumley, L.M., and F.A.H. Sperling. 2011b. Life-history traits maintin the genomic integrity of sympatric species of the spruce budworm (Choristoneura fumiferana) group on an isolated forest island). Ecology and Evolution, 1: 119-131.

Lysyk, T.J. 1988. A multiple-cohort model for simulating jack pine budworm (Lepidoptera: Tortricidae) development under variable temperature conditions. The Canadian Entomologist, 121: 373–387.

Lysyk, T.J. 1989. Stochastic model of eastern spruce budworm (Lepidoptera: Tortricidae) phenology on white spruce and balsam fir. Journal of Economic Entomology, 82: 1161-1168.

MacLean, D.A., Erdle, T.A., MacKinnon, W.E., Porter, K.B., Beaton, K.P., Cormier, G., Morehouse, S., and M. Budd. 2001. The spruce budworm decision support system: forest

protection planning to sustain long-term wood supply. Canadian Journal of Forest Research, 31: 1742-1757.

Mallet, J. 1995. A species definition for the Modern Synthesis. Trends in Ecology and Evolution, 10: 294-299.

Mallet, J. 2001. The speciation revolution. Journal of Evolutionary Biology, 14: 887-888.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution, 20: 229-237.

Mallet, J. 2007. Hybrid speciation. Nature, 446: 279-283.

Martin, C.H., and L.C. Feinstein. 2014. Novel trophic niches drive variable progress towards ecological speciation within an adaptive radiation of pupfishes. Molecular Ecology, 23: 1846-1862.

Martin, N.H., Bouck, A.C., and M.L. Arnold. 2006. Detecting adaptive trait introgression between Iris fulva and I. brevicaulis in highly selecti*ve field conditions. Genetics, 172: 2481-2489.

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Pinero, D., and B.C. Emerson. 2014. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for populations genetic inference. Molecular Ecology resources, doi:10.111/1755-0998.12291.

Matute, D.R., Butler, I.A., Turissini, D.A., and J.A. Coyne. 2010. A test of the snowball theory for the rate of evolution of hybrid incompatibilities. Science, 329: 1518-1521.

Mayr, E. 1969. The biological meaning of species. Biological Journal of the Linnean Society, 1: 311-320.

McGugan, B.M. 1954. Needle-mining habits and larval instars of the spruce budworm. The Canadian Entomologist, 86: 439-454.

Meglecz, E. 2007. MicroFamily: A computer program for detecting flanking region similarities among different microsatellite loci. Molecular Ecology Notes, 7: 18-20.

Michel, A.P., Sim, S., Powell, T.H.Q., Taylor, M.S., Nosil, P., and J.L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences, 107: 9724-9729.

Miller, C.A. 1958. The measurement of spruce budworm populations and mortality during the first and second larval instars. The Canadian Jounral of Zoology, 36: 409-422.

Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A., and E.A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research, 17: 240-248.

Milne, R.I., and R.J. Abbott. 2008. Reproductive isolation among two interfertile Rhododendron species: low frequency of post-F1 hybrid genotypes in alpine hybrid zones. Molecular Ecology, 17: 1108-1121.

Minder, A.M., and A. Widmer. 2008. A population genomic analysis of species boundaries: neutral processes, adaptive divergence and introgression between two hybridizing plant species. Molecular Ecology, 17: 1552-1563.

Miner, B.G, Sultan, S., Morgan, S.G., Padila, D.K., and R.A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution, 20: 685-692.

Morjan, C.L., and L.H. Rieseberg. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Molecular Ecology, 13: 1341-1356.

Muir, G., and C. Schlotterer. 2005. Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiationg two hybridizing oaks (Quercus spp.). Molecular Ecology, 14: 549-561.

Nadeau, N.J., Martin, S.H., Kozak, K.M., Salazar, C., Dasmahapatra, K.K., Davey, J.W., Baxter, S.W., Blaxter, M.L., Mallet, J., and C.D. Jiggins. 2013. Genome-wide patterns of divergence and gene flow across a butterfly radiation. Molecular Ecology, 22: 814-826.

Nealis, V.G. 2005 Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera: Tortricidae) and their hybrid progeny. The Canadian Entomologist, 137: 584–597.

Nealis, V.G. 2008. Spruce budworms, Choristoneura Lederer (Lepidoptera: Tortricidae). Pp. 3524-3531 in J.L. Capinera (Ed.), Encyclopedia of Entomology, 2nd edition. Springer, Dordrecht. Pp. 4411.

Nealis, V.G., and J. Regniere. 2009. Risk of dispersal in western spruce budworm. Agricultural and Forest Entomology, 11: 213-223.

Nolte, A.W., and D. Tautz. 2010.Understanding the onset of hybrid speciation. Trends in Genetics, 26: 54-58.

Nolte, A.W., Gompert, Z., and C.A. Buerkle. 2009. Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. Molecular Ecology, 18: 2615-2627.

Noor, M.A.F. 2002. Is the biological species concept showing its age. Trends in Ecology and Evolution, 17: 153-154.

Nosil, P. 2012. Ecological Speciation. Oxford University Press, University of Oxford, Oxford, United Kindom. 280pp.

Nosil, P., and J.L. Feder. 2012. Genomic divergence during speciation: causes and consequences. Philosophical Transactions of the Royal Society B, 367: 332-342.

Nosil, P., and S.M. Flaxman. 2011. Conditions for mutation-order speciation. Proceeding of the Royal Society of London B, 278: 399-407.

Nosil, P., Funk, D.J., and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology, 18: 375-402.

Nosil, P., Gompert, Z., Farkas, T.E., Comeault, A.A., Feder, J.L., Buerkle, C.A., and T.L. Parchman. 2013. Genomic consequences of multiple speciation processes in a stick insect. Proceedings of the Royal Society of London B, 279: 5058-5065.

Nosil, P., Vines, T.H., and D.J. Funk. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution, 59: 705-719.

Orr, H.A., and M. Turelli. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution, 55: 1085-1094.

Parchman, T.L., Gompert, Z., Braun, M.J., Brumfield, R.T., McDonald, D.B., Uy, J.A.C., Jarvis, E.D., Schlinger, B.A., and C.A. Buerkle. 2013. The genomic consequences of adaptive divergence and reproductive divergence and reproductive isolation between species of manakins. Molecular Ecology, 22: 3304-3317.

Park, S. D. E. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. Ph.D. thesis, University of Dublin.

Parsons, T.J., Olson, S.L., and M.J. Braun. 2006. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. Science, 260: 1643-1646.

Payseur, B.A. 2010. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. Molecular Ecology Resources, 10: 806-820.

Peakall, r., and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Populations genetic software for teaching and research-an update. Bioinformatics, 28: 2537-2539.

Pertea, G., Huang, X., Liang, F., Antonescu, V., Sultana, R., Karamycheva, S., Lee, Y., White, J., Cheung, F., Parvizi, B., Tsai, J., and J. Quackenbush. 2002. TIGR Gene Indices Clustering Tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics, 19: 651-652.

Petit, R.J., and L. Excoffier. 2009. Gene flow and species delimitation. Trends in Ecology and Evolution, 24: 386-393.

Poland, J.A., Brown, P.J., Sorrells, M.E., and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLOS One 7:e32253. doi:10.1371/journal.pone.0032253.

Porter, A.H., Wenger, R., Geiger, H., Scholl, A., and A.M. Shapiro. 1997. The Pontia daplidice-edusa hybrid zone in Northwestern Italy. Evolution, 51: 1561-1573.

Powell, J.A., and J.A. DeBenedictis. 1995. Evolutionary interpretations, taxonomy, and nomenclature. Pp. 217-275 in J.A. Powell (Ed.), Biosystematic studies of conifer-feeding Choristoneura (Lepidoptera: Tortricidae) in the western United States. Univesity of California Press, Entomology Volume 115, Berkeley, California. pp. 275.

Powell, T.H.Q., Hood, G.R., Murphy, M.O., Heilveil, J.S., Berlocher, S.H., Nosil, P., and J.L., Feder. 2013. Genetic divergence along the speciation continuum: the transition from host race to species in Rhagoletis (Diptera: Tephritidae). Evolution, 67: 2561-2576.

Pringle, E.G., Baxter, S.W., Webster, C.L., Papanicolaou, A., Lee, S.F., and C.D. Jiggins. 2007. Synteny and chromosome evolution in the Lepidoptera: Evidence from mapping in Heliconius Melpomene. Genetics, 177: 417-426.

Pritchard, J.K., Stephens, M., and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Pruett, C.L., and K. Winker. 2008. The effects of sample size on populations genetic diversity estimates in song sparrows *Melospiza melodia*. Journal of Avian Biology, 39: 252-256.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rasanen, K., and A.P. Hendry. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. Ecology Letters, 11: 624-636.

Rastas, P., Paulin, L., Hanski, I., Lehtonen, R., and P. Auvinen. 2013. Lep-MAP: fast and accurate linkage map construction for large SNP datasets. Bioinformatics, 29: 3128-3134.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86: 248-249.

Razowski, J. 2008. Tortricidae (Lepidoptera) from South Africa. 6: Choristoneura Hubner and Procrica Diakonoff. Polish Journal of Entomology, 77: 245-254.

Regniere, J., and R.M. Fletcher. 1983. Direct measurement of spruce budworm (Lepidoptera: Tortricidae) larval dispersal in forest stands. Environmental Entomology, 12: 1532-1538.

Regniere, J., Delisle, J., Pureswaran, D.S., and R. Trudel. 2012. Mate-finding allee effect in spruce budworm ppulation dynamics. Entomologia Experimentalis et Applicata, 146: 112-122.

Renaut, S., Maillet, N., Normandeau, E., Sauvage, C., Derome, N., Rogers, S.M., and L. Bernatchez. 2012. Genome-wide patterns of divergence during speciation: the lake whitefish case study. Philosophical transactions of the Royal Society B, 367: 354-363.

Rieseberg, L.H. 1997. Hybrid origins of plant species. Annual review of Ecology, Evolution, and Systematics, 28: 359-89.

Rieseberg, L.H., and J.M. Burke. 2001. A genic view of species integration. Journal of Evolutionary Biology, 14: 883-886.

Rieseberg, L.H., Atrcher, M.A., and R.K. Wayne. Transgressive segregation, adaptation and speciation. Heredity, 83: 363-372.

Robert, L.-E., Kneeshaw, D., and B.R. Sturtevante. 2012. Effects of forest management legacies on spruce budworm (Choristoneura fumiferana) outbreaks. Canadian Jounral of Forest Research, 42: 463-475.

Roe, A.D., and F.A.H. Sperling. 2007. Population structure and species boundary delimitation of cryptic Dioryctria moths: an integrative approach. Molecular Ecology, 16: 3617–3633.

Rogers, M.E., Krieger, J., and R.G. Vogt. 2001. Antennal SNMPs (Sensory Neuron Membrane Proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. Journal of Neurobiology, 49: 47-61.

Rousset, F. 2008. GENEPOP'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8: 103-106.

Rowe, H.C., Renaut, S., and A. Guggisberg. 2011. RAD in the realm of next-generation sequencing technologies. Molecular Ecology, 20: 3499-3502.

Rozen, S., and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S. (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp. 365-386.

Rundle, H.D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. Evolution, 56: 322-329.

Rundle, H.D., and M.C. Whitlock. 2001. A genetic interpretation of ecologically dependent isolation. Evolution, 55: 198-201.

Rundle, H.D., and P. Nosil. 2005. Ecological speciation. Ecology letters, 8: 336-352.

Sanders, C.J. 1971a. Daily activity patterns and sex pheromone specificty as sexual isolating mechanisms in two species of Choristoneura (Lepidoptera: Tortricidae). The Canadian Entomologist, 103: 498-502.

Sanders, C.J. 1971b. Sex pheromone specificity and taxonomy of budworm moths (Choristoneura). Science, 171: 911-913.

Sanders, C.J. 1991. Biology of North American spruce budworm. Pp. 579–620, in L.P.S. van der Gesst and H.H. Evenhuis (Eds.), Tortricid pests, their biology, natural enemies and control. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Sanders, C.J., Daterman, G.E., and T.J. Ennis. 1977. Sex pheromone responses of Choristoneura spp. and their hybrids (Lepidoptera: Tortricidae). The Canadian entomologist, 109: 1203-1220.

Scascitelli, M., Whitney, K.D., Randell, R.A., King, M., Buerkle, C.A., and L.H. Rieseberg. 2010. Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H. debilis*) reveals asymmetric patterns of introgression and small islands of genomic differentiation. Molecular Ecology, 19: 521-541.

Schmidt, F.H. and W.L. Lauer. 1977. Developmental polymorphism in Choristoneura spp. (Lepidoptera: Tortricidae). Annals of the Entomological Society of America, 70: 112-118.

Schmidt, F.H., Campbell, R.K., and S.J. Trotter, Jr. 1977. Errors in determining instar numbers through head capsule measurements of a lepidopteran - a laboratory study and critique. Annals of the Entomological Society of America, 70: 750-756.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology, 18: 233-234.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution, 19: 198-207.

Servedio, M.R., and M.A.F. Noor. 2003. The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution, and Systematics, 34: 339-364.

Shepherd, R.F. 1985. A theory on the effects of diverse host-climatic environments in British Columbia on the dynamics of western spruce budworm. In Recent advances in spruce budworms research. Proceedings of the CANUSA spruce budworms research symposium, Bangor, Maine, 16-20 September, 1984. Edited by C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy. Canadian Forestry Service, Ottawa, Ontario, Canada. Pp. 60–70.

Shepherd, R.F., Gray, T.G., and G.T. Harvey. 1995. Geographical distribution of Choristoneura species (Lepidoptera: Tortricidae) feeding on Abies, Picea, and Pseudotsuga in western Canada and Alaska. The Canadian Entomologist, 127: 813-830.

Shriver, M.D., Smith, M.W., Marcini, A., Akey, J.M., Deka, R., and R.E. Ferrell. 1997. Ethnic-affiliation estimation by use of population-specific DNA markers. American Journal of Human Genetics, 60: 957-964.

Sichova, J., Nyugen, P., Dalikova, M., and F. Marec. 2013. Chromosomal evolution in Tortricid moths: conserved karyotypes with diverged features. PLoS One, 8(5) e64520. Doi:10.1371/journal.pone.0064520.

Sinama, M., Dubut, V., Costeboat, C., Gilles, A., Junker, M., Malausa, T., Martin, J.-F., Neve, G., Pech, N., Schmitt, T., Zimmermann, M., and E., Meglecz. 2011. Challenges of microsatellite development in Lepidoptera: Euphydras aurinia (Nymphalidae) as a case study. European Jounral of Entomology, 108: 261-266.

Smith, K.L., Hale, J.M., Kearney, M.R., Austin, J.J., and J. Melville. 2013. Molecular patterns of introgression in a classic hybrid zone between the Australian tree fogs, Litoria ewingii and L. paraewingi: evidence of a tension zone. Molecular Ecology, 22: 1869-1883.

Smith, S.G. 1944. The reproduction of the nucleus. Scientific Agriculture, 25: 566-571.

Smith, S.G. 1953. Reproductive isolation and the integrity of two sympatric species of Choristoneura (Lepidoptera: Tortricidae). The Canadian Entomologist, 85: 141-151.

Smith, S.G. 1954. A partial breakdown of temporal and ecological isolation between Choristoneura species (Lepidoptera: Tortricidae). Evolution, 8: 206-224.

Sonah, H., Bastien, M., Iquira, E., Tardive, A., Legare, G., Boyle, B., Normandeau, E., Laroche, J., Larose, S., Jean, M., and F. Belzile. 2013. An imporved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PLOS One, 8:e54603. doi:10.1371/journal.pone.0054603

Sperling, F.A.H. 1994. Sex-linked genes and species differences in Lepidoptera. The Canadian Entomologist, 126: 807-818.

Sperling, F.A.H. 2003. Butterfly Molecular Systematics: from species defintions to higher-level phylogenies. Pp. 431-458 in C.L. Boggs, W.B. Watt, and P.R. Ehrlich (Eds.), Butterflies: Ecology and Evolution taking flight. University of Chicago Press.

Sperling, F.A.H., and D.A. Hickey. 1994. Mitochondrial DNA sequence variation in the spruce budworm species complex (Choristoneura: Lepidoptera). Molecular Biology and Evolution, 11: 656-665.

Sperling, F.A.H., and D.A. Hickey. 1995. Amplified mitochondrial DNA as a diagnostic marker for species of conifer-feeding Choristoneura (Lepidoptera: Tortricidae). The Canadian Entomologist, 127: 277-288.

Stehr, G.W. 1967. On coniferophagous species of Choristoneura (Lepidoptera: Tortricidae) in North America, II. Geographic distribution in accordance with forest regions. The Canadian Entomologist, 99: 456-463.

Stolting, K.N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. , and C. Lexer. 2013. Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. Molecular Ecology, 22: 842-855.

Strasburg, J.L., Sherman, N.A., Wright, K.M., Moyle, L.C., Willis, J.H., and L. Rieseberg. 2012. What can patterns of differentiation across plant genomes tell us about adaptation and speciation. Philosophical Transactions of the Royal Society B, 367: 364-373.

Strong, W.L., and L.V. Hills. 2006. Taxonomy and origin of present-day morphometric variation in Picea glauca (x engelmannii) seed-cone scales in North America. Canadian Journal of Botany, 84: 1129–1141.

Suomalainen, E., Cook, L.M., and J.R.G. Turner. 1973. Achiasmatic oogenesis in the Heliconiine butterflies. Hereditas, 74: 302-304.

Szymura, J.M., and N.H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, Bombina bombina and B. variegata, near Cracow in southern Poland. Evolution, 40: 1141-1159.

Szymura, J.M., and N.H. Barton. 1991. The genetic structure of the hybrid zone between the fire-bellied toads Bombina bombina and B. variegata: comparisons between transects and between loci. Evolution, 45: 235-261.

Teeter, K.C., Payseur, B.A., Harris, L.W., Bakewell, M.A. Thibodeau, L.M., O'Brien, J.E., Krenz, J.G., Sans-Fuentes, M.A., Nachman, M.W., and P.K. Tucker. 2008. Genome-wide patterns of gene flow across a house mouse hybrid zone. Genome Research, 18: 67-76.

Teeter, K.C., Thibodeau, L.M., Gompert, Z., Buerkle, C.A., Nachman, M.W., and P.K. Tucker. 2010. The variable genomic architecture of isolation between hybridizing species of house mice. Evolution, 64: 472-485.

Thomson, A.J., Shepherd, R.F., Harris, J.W.E., and R.H. Silversides. 1984. Relating weather to outbreaks of western spruce budworm, Choristoneura occidentalis (Lepidoptera: Tortricidae), in British Columbia. The Canadian Entomologist, 116: 375–381.

Tobler, A., Kapan, D., Flanagan, N.S., Gonzalez, C., Peterson,E., Jiggins, C.D., Johnston, S., Heckel, D.G., and W.O. McMillan. 2005. First generation linkage map of the warningly colored butterfly Heliconius erato. Heredity, 94: 408-417.

Turner, T.L., Hahn, M.W., and S.V. Nuzhdin. 2005. Genomic islands of speciation in Anopheles gambiae. PLoS Biology, 3(9): e285.

Van Dongen, S., Backeljau, T., Matthysen, E., and A.A. Dhondt. 1998. Genetic population structure of the winter moth (*Operophtera brumata* L.) (Lepidoptera, Geometridiae) in a fragmented landscape). Heredity, 80: 92-100.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535-538.

Van't Hof, A.E., Brakefield, P.M., Saccheri, I.J., and B.J. Zwaan. 2007. Evolutionary dynamics of multilocus microsatellite arrangements in the genome of the butterfly Bicyclus anynana, with implications for other Lepidoptera. Heredity, 98: 320-328.

Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B, 367: 451-460.

Via, S., and J. West. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Molecular Ecology, 17: 4334-4345.

Vines, T.H., Köhler, S.C., Thiel, M., Ghira, I., Sands, T.R., MacCallum, C.J., Barton, N.H., and B. Nürnberger. 2003. The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied taods Bombina bombina and B. variegate. Evolution, 57: 1876-1888.

Volney, W.J.A. 1985. Comparative population biologies of North American spruce budworms. Pp. 71-84 in C.J. Sanders, R.W. Stark, E.J. Mullins, and J. Murphy (Eds.), Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworm

Research Symposium, Bangor, Maine, 16-20 September 1984. Bangor, Maine. Supply and Services, Ottawa, Canada. 527 pp.

Volney, W.J.A. 1999. Genetic and ecological controls of post-diapause development in Choristoneura. In Physiology and genetics of tree-phytophage interactions, Gujan, France, August 31 to September 5, 1997. Edited by F. Lieutier, W.J. Mattson, and M.R. Wagner. Institut National de la Recherche Agronomique (INRA), Paris, France. Pp. 187–195.

Volney, W.J.A., and H.F. Cerezke. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. Canadian Journal of Forest Research, 22: 198–205.

Volney, W. J. A., and R.A. Fleming. 2000. climate change and impacts of boreal forest insects. Agriculture, Ecosystems and Environment, 82: 283-294.

Volney, W. J. A., and R.A. Fleming. 2007. Spruce budworm (Choristoneura spp.) biotype reactions to forest and climate characteristics. Global Change Biology, 13: 1630-1643.

Volney, W.J.A., and A.M. Liebhold. 1985. Post-diapause development of sympatric Choristoneura occidentalis and C. retiniana (Lepidoptera: Tortricidae) and their hybrids. The Canadian Entomologist, 117: 1479-1488.

Volney, W.J.A., Liebhold, A.M., and W.E. Waters. 1984. Host associations, phenotypic variation, and mating compatibility of Choristoneura occidentalis and C. retiniana (Lepidoptera: Tortricidae) populations in south-central Oregon. The Canadian Entomologist, 116: 813-826.

Volney, W.J.A, Waters, W.E., Akers, R.P., and A.M. Liebhold. 1983. Variation in spring emergence patterns among western Choristoneura spp. (Lepidoptera: Tortricidae) populations in southern Oregon. The Canadian Entomologist, 115: 199-209.

Wang, B., and A.H. Porter. 2004. An AFLP-based interspecific linkage map of sympatric hybridizing Colias butterflies. Genetics, 168: 215-225.

Wang, R.J., Ane, C., and B.A. Payseur. 2013. The evolution of hybrid inompatibilities along a phylogeny. Evolution, 67: 2905-2922.

White, J. R., Roberts, M., Yorke, J. A., and M. Pop. 2008. Figaro: a novel statistical method for vector sequence removal. Bioinformatics, 15: 462-467.

Wu, C.-I. 2001. The genic view of the process of speciation. Journal of Evolutionary Biology, 14: 851-865.

Yamamoto, S., and T. Sota. 2012. Parallel allochronic divergence in a winter moth due to disruption of reproductive period by winter harshness. Molecular Ecology, 21: 174-183.

Yammamoto, K., Nohata, J., Kadono-Okuda, K., Narukawa, J., Sasanuma, M., Sasanuma, S., Minami, H., Shimomura, M., Suetsugo, Y., Banno, Y., Osoegawa, K., de Jong, P.J., Goldsmith, M.R., and K. Mita. 2008. A BAC-based integrated linkage map of the silkworm Bombyx mori. Genome Biology, 9: R21. Doi:10.1186/gb-2008-9-1-r21.

Yasukochi, Y., Ashakumary, L.A., Baba, K., Yoshida, A., Sahara, K. 2006. A second generation integrated map of the silkworm reveals synteny and conserved gene order between lepidopteran insects. Genetics, 173: 1319-1328.

Zane, L., Bargelloni, L., and T. Patarnello. 2002. Strategies for microsatellite isolation: a review. Molecular Ecology, 11: 1-16.

Zhang, D. X. 2004. Lepidopteran microsatellite DNA: redundant but promising. Trends in Ecology and Evolution, 19: 507-509.

Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C., and B.S. Weir. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics, 28: 3326-3328.

Zhulidov, P.A., Bogdanova, E.A., Shcheglov, A.S., Vagner, L.L., Khaspekov, G.L., Kozhemyako, V.B., Matz, M.V., Meleshkevitch, E., Moroz, L.L., Lukyanov, S.A., and D.A. Shagin. 2004. Simple cDNA normalization using kamchatka crab duplex-specific nuclease. Nucleic Acids Research, 32:e37. doi:10.1093/nar/gnh031.