Speciation and hybridization in the Old World swallowtail butterfly (*Papilio machaon*) species complex

by

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Abstract

Species delimitation is fundamental to evolutionary biology. However the process is far from straightforward in systems with complex evolutionary histories, and the concept of species as taxonomic hypotheses is often overlooked in many biological disciplines. Here I investigate species delimitation operationally, with a review and meta-analysis of the literature, and empirically, by investigating hybridization in swallowtail butterflies. First, I conducted a literature review on studies that used multiple molecular markers to delimit closely related species of animals and fungi. I evaluated the relative success of different types of molecular markers (mitochondrial, ribosomal, nuclear, and sex-linked genes) in delimiting closely related species and asked whether increased geographic or population-level sampling and the number of markers affected identification success. With this foundation, I then investigated hybridization in the Old World swallowtail butterfly (*Papilio machaon* Linnaeus, 1758) species complex. At a North America-wide scale, I assessed the putative hybrid origins of multiple lineages in the group, using morphology, mitochondrial DNA, microsatellites, and ecological characteristics. I then focused on a hybrid zone in southwestern Alberta and tested whether population genetic structure of the area (using mitochondrial DNA and microsatellites) was similar to an assessment done 30 years ago using morphology and allozymes. I also compared multiple hybrid identification and classification (F1, F2, backcross) methods for microsatellites and a genome-wide single nucleotide polymorphism dataset for a subset of individuals. Finally, I asked whether environmental or landscape variables could explain variation in genetic differentiation and interspecific hybridization in this hybrid zone, using spatial ecology and landscape genetics methods. This is the first application of raster-based landscape genetics methods to interspecific hybridization. Together, the progression of studies in this thesis provide important insight into species delimitation and add to a growing body of research documenting the complexity of hybridization, as well as its potential for generating biodiversity.

Preface

This thesis is an original work by Julian R Dupuis. However, productive science is achieved through collaborations and mentorship; therefore, "we" is used throughout the text of the data chapters as a reflection of those involved.

A version of chapter 2 has been published as Dupuis, JR, Roe AD, Sperling FAH (2012) "Multi-locus species delimitation in closely related animals and fungi: one marker is not enough." Molecular Ecology volume 21, pages 4422-4436. Here I collected data and conceived and conducted analyses, and drafted the original manuscript. ADR helped to collect data and conceive analyses, and FAHS contributed conceptual guidance. All authors contributed analytical guidance and provided input to the manuscript throughout its preparation.

A version of chapter 3 has been published as Dupuis JR, Sperling FAH (2015) "Repeated reticulate evolution in North American *Papilio machaon* group swallowtail butterflies." PLOS ONE volume 10, e0141882. Here I collected data, conducted data analyses, and drafted the original manuscript, and FAHS contributed conceptual guidance. All authors contributed analytical guidance and provided input to the manuscript throughout its preparation.

In memory of my father, Earl Bradley Dupuis

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Chapter 1

General Introduction

1.1 General introduction

Ever since the ancient Greeks, species have been considered the fundamental unit of life, the most basic "kind" of biological entity, based on inherent properties that distinguish one species from another (typological species: Ruse 1969; Mishler 2010). Several other fundamental units are now recognized in the life sciences (cells: Mozzarello 1999, genes: Koonin & Wolf 2009), but species remain fundamental to our understanding of the biological world. However, our current understanding of "species" differs greatly from the concept of typological biological entities. The past century and a half has borne witness to the development of more complex views of what "species" represent and how they should be defined. The Darwinian "revolution" commenced this change in viewpoint (but see Hodge 2005 and Ruse 2009), with the idea that species were natural genealogical units, sharing inheritance from common ancestors. From those pioneering ideas about evolution came the development of myriad species concepts focusing on groups of interbreeding organisms (the biological species concept: Mayr 1942), overall similarity (the phenetic species concept: Sokal & Crovello 1970), phylogenetic groupings (the phylogenetic species concept: Cracraft 1983), ecological niches (the ecological species concept: Van Valen 1976), evolutionary roles (the evolutionary species concept: Simpson 1951), and genotypic clusters (the genotypic cluster species concept: Mallet 1995). These concepts focus on varied conditions that may be appropriate for characterizing diverging lineages in specific groups of organisms. However, taken together, their diversity highlights one of the largest difficulties in answering the question "what is a species?", which lies in how species themselves are delimited

(De Queiroz 2007). Without a clear, methodological framework for how species boundaries are determined, general conceptualization of species categories is less meaningful.

Unfortunately, species delimitation is often far from straightforward. Retained ancestral polymorphism, incomplete lineage sorting, and gene tree incongruence are common evolutionary phenomena, and can introduce uncertainty in demarcation of species boundaries (Sites & Marshall 2004, Knowles & Carstens 2007). Hybridization between divergent lineages or populations can also create complicated evolutionary histories through its reticulated nature. Although early evolutionary biologists discounted the role of hybridization in the overall diversification of animal life (e.g. Wagner 1970), we now recognize that hybridization is common in animals and can have highly diverse evolutionary outcomes (Mallet 2007, Nolte & Tautz 2010, Butlin *et al.* 2012, Harrison 2012, Abbott *et al.* 2013). Hybridization can homogenize lineages via neutral diffusion (Hewitt 1988, Taylor *et al.* 2006), but also produce novel adaptive characteristics that facilitate diversification of new lineages (Rieseberg *et al.* 2003, Mallet 2007, Jiggins *et al*. 2008). Additionally, these processes can occur at both small and large genomic scales (Dasamahapatra *et al.* 2012, Rieseberg *et al.* 2003), making even the detection of hybridization a nontrivial task. The varied outcomes of hybridization make it a wildcard to the process of species delimitation, supporting the need for research investigating dynamics of the phenomenon at different evolutionary scales.

Swallowtail butterflies of the *Papilio machaon* species group (Lepidoptera: Papilionidae) provide a rich foundation to study evolutionary biology. Largely owing to their relatively large size (wingspan ~5-9 cm) and charismatic black and yellow coloration, these butterflies have piqued the interest of amateur and professional biologists for centuries (e.g. Linnaeus 1758, Fabricius 1775, Stoll 1782, Lucas 1852). This long history combined with myriad ecological and geographic races and color morphs has fueled extensive debate surrounding the systematic relationships (e.g. Ae 1979, Clarke & Larsen 1986) and species limits of the group (Clarke & Sheppard 1955, Sperling 1987, Sperling 2003). Six species are currently recognized in North America: *Papilio brevicauda* Saunders, 1868 *,* *P. indra* Reakirt, 1866, *P. joanae* Heitzman, 1973*, P. machaon*, *P. polyxenes* Fabricius, 1775, and *P. zelicaon* Lucas, 1852 (Pelham 2008). *Papilio machaon* is the only member of the group with a Holarctic distribution, and extends from western United States into Russia and west throughout Europe. Other species are restricted to the New World (Figure 3.1). Larvae mimic bird droppings during early instars, and develop alternating black and green bands with yellow or red spots in later instars. Their host plants generally include members of the families Apiaceae (Umbelliferae) and Asteraceae (Compositae), although Rutaceae is used locally by *P. zelicaon* (Scott 1986, Sperling 1987, Sperling 1990). *Papilio indra* is the only member of the group with consistently different genitalia and adult wing morphology (Sperling & Harrison 1994), and is considered basal to the remainder of the group (Emmel & Emmel 1964, Condamine *et al.* 2012).

1.2 Thesis overview

In this thesis, I address the evolutionary history and hybridization dynamics of the *Papilio machaon* species group in North America. Chapters progress from broad- to fine-scale; I address hybrid origins and evolutionary relationships of multiple lineages in North America (chapter 3), then focus on finer-scale hybrid interactions in southwest Alberta (chapters 4 and 5). However, any research into evolutionary history, and particularly reticulate evolutionary history, is implicitly tied to the biological entities in question, namely “species”. To provide a conceptual foundation to research questions on swallowtail butterflies, I first conduct a meta-analysis/review on species delimitation using molecular markers (chapter 2).

A fundamental, but neglected, concept in biology is that species are hypotheses. Although often treated as concrete “facts” of the natural world, in reality species are testable taxonomic hypotheses of the dynamic relationships of biodiversity (Pante *et al.* 2015). This disconnection between taxonomy and other biological disciplines that use species designations is epitomized by efforts to simplify the process of species delimitation into automated or semi-automated systems. DNA barcoding is one such effort, which originally aimed to establish a global bioidentification system based on a single, mitochondrial DNA marker (Hebert *et al.* 2003, Hajibabaei *et al.* 2007, Ratnasingham & Hebert 2007). Early DNA barcoding studies reported high success in species identification (96-100%, Hebert *et al.* 2003, Barrett & Hebert 2005, Janzen *et al*. 2005). However, these studies generally had a small geographic focus and limited sample sizes, and masked lower success in delimiting closely related species by including divergent taxa (where it is generally unnecessary to have a DNA-based identification system). Other studies addressing DNA barcoding methodology reported much lower success (e.g. 40%: Whitworth *et al.* 2007; <70%: Meier *et al.* 2006; 58-84%: Wiemers & Fiedler 2007), and many postulated that increased population-level or geographic sampling identifies more variation and leads to lower identification success (Moritz & Cicero 2004, Meier 2008). To avoid potential bias in identification success rate by the inclusion of divergent taxa, in **chapter 2** I conducted a review and meta-analysis of studies that used multiple molecular markers to delimit closely related species (generally recently-diverged sister-species). I also investigated whether increased geographic or population-level sampling and the number of markers affected identification success. These considerations are pivotal to providing objective assessment of the success of

DNA-based identification systems, which are increasingly being used in both systematic and applied settings (e.g. Kõljalg *et al.* 2013, Wells & Škaro 2014).

Hybridization is increasingly recognized as an important force in generating biodiversity through both adaptive introgression (Hedrick 2013) and hybrid speciation (Rieseberg *et al.* 2003, Mallet 2007, Jiggins *et al*. 2008). Although both of these processes are initiated through hybridization, they represent conceptually distinct evolutionary processes. In adaptive introgression, hybridization passes adaptive traits or variability from one species to another; this adaptive variability replaces original genomic characteristics but leaves the majority of the genome intact (Whitney *et al.* 2010, Pardo-Diaz *et al.* 2012, Hedrick 2013). Alternatively, in cases of homoploid hybrid speciation, hybridization creates unique combinations of parental traits in distinct and independent hybrid progeny, which are reproductively isolated from their parents (Gross & Rieseberg 2005, Mallet 2007, Dasmahapatra *et al.* 2012, Abbott *et al.* 2013). The distinction between these processes is narrow, but is important when species designations of putative hybrids are used for management (DeMarias *et al.* 1992, Haig *et al.* 2004, Monzón *et al.* 2014). Additionally, most measures of genomic admixture, although conceptually appealing to describe hybrids, are insufficient to distinguish these processes (Abbott *et al.* 2013). In **chapter 3**, I characterized hybridization in the *P. machaon* species group across North America using morphology, mitochondrial DNA (mtDNA), microsatellite markers, and ecological characteristics. I focused on four putative hybrid populations that were identified in previous research as having unexpected mtDNA signatures (Sperling & Harrison 1994). By using multiple data types to infer parental characteristics of each hybrid lineage, I differentiated between adaptive introgression and hybrid speciation, and used molecular dating to infer the phylogeographic history of these lineages.

Genetic surveys of population structure are a valuable tool to assess hybrid interactions, particularly in hybrid zones where parental and hybrid individuals may be found in the same geographic region. The evolutionary outcomes of hybrid zones are variable. Neutral diffusion can homogenize populations, thus inhibiting speciation (Hewitt 1988, Taylor *et al.* 2006, Harrison 2012). Tension zones can form when selection against hybrids limits neutral introgression between parental species (Barton & Hewitt 1985). If selection is strong, reduced hybrid fitness can reinforce premating barriers, thus advancing speciation (Servedio & Noor 2003, Via 2009), but if selection is weak, tension zones can stabilize, thereby maintaining differentiation but stalling speciation (e.g. Ruegg 2008). When parental species are parapatric, tension zones often form in linear configurations, grading from one parent to another and facilitating cline based analyses to characterize hybrid zone structure (e.g. Barton & Hewitt 1985, Teeter *et al.* 2009). However, in hybrid interactions with widely distributed or sympatric parental species, mosaic hybrid zones (Harrison & Rand 1989) may form and be more difficult to characterize using traditional cline based analyses.

Characterization of hybrid zones most often relies on classifications based on early plant and animal breeding experiments: parental, filial (F1, F2, etc.), and backcross (e.g. Bateson & Saunders 1902). These classifications can be quantified into a hybrid index, where either parental species is assigned a value of zero or one, and F1 hybrids a value of 0.5. Although widespread in the literature and appealing in its simplicity, this method for classifying hybrids may ignore genomic characteristics that differentiate later generation hybrids and backcrosses (Jiggins & Mallet 2000, Fitzpatrick 2012), and more sophisticated model-based methods for hybrid classification have proliferated (e.g. Anderson & Thompson 2002, Buerkle 2005, Gompert & Buerkle 2010, Fitzpatrick 2012). Additionally, the type and number of genetic markers used to characterize hybrids can greatly affect conclusions regarding hybrid zone structure (Fitzpatrick 2012).

In southwestern Alberta, hybrid individuals between *P. machaon* and *P. zelicaon* have been documented since the early 1900's (Sperling 1987), and in **chapter 4**, I used a population genetics approach by surveying geographic variation in mtDNA, microsatellites, and genome-wide single nucleotide polymorphisms (for a subset of individuals) to characterize this hybrid zone. I tested whether similar hybrid signatures were apparent, compared to an assessment using morphology and allozymes 30 years ago (Sperling 1987, Sperling 1990). Again, making use of multiple datasets, I compared methods for identifying hybrid individuals and classifying them (F1, F2, backcross, etc.), and compared hybrid signatures between genetic markers.

Research on hybridization has made extensive use of genomic tools to study endogenous factors affecting hybrid interactions (e.g. hybrid viability, fecundity, genomic mosaicism, etc.: Teeter *et al.* 2009, Abbott *et al.* 2013, Rieseberg *et al.* 2003, Dasmahapatra *et al.* 2012, Harrison & Larson 2014). Exogenous factors (environmental, landscape-based, etc.) also affect hybrid dynamics (Barton & Hewitt 1985, Harrison & Rand 1989), but have seen less integration with modern methods, such as landscape genetics. Landscape genetics combines population genetics with spatial ecology, and investigates how environmental or landscape characteristics affect gene flow between populations (Manel *et al.* 2003, Storfer *et al.* 2007, Manel & Holderegger 2013). This effect is often quantified using raster-based landscape data via geographic information systems (GIS). While hybrid zone research often integrates aspects of the landscape or environment in explaining hybrid occurrence (e.g. Fitzpatrick & Shaffer 2007, Pfennig 2007, Larson *et al.* 2013), this is generally limited to association-based analyses, rather than those considering spatial heterogeneity at the landscape scale. Multiple levels of genetic differentiation in hybrid systems (i.e. between parental species, parents and hybrids, and between hybrids), combined with the fact that different measures of genetic differentiation and diversity often lead to different conclusions in landscape genetics studies (Storfer *et al.* 2010, Pérez-Espona *et al.* 2012, Keller *et al.* 2013, Zancolli *et al.* 2014), lead to highly complicated applications of landscape genetics to hybrid systems. In **chapter 5**, I used environmental and landscape variables to explain the varying amounts of genetic differentiation between *P. machaon* and *P. zelicaon* across Alberta, as well as the hybridization characterized in chapter 4. I used multiple statistical methods, and present the first use of raster-based landscape genetics methods in a hybrid system. By focusing on hierarchical spatial scales, I was able to simplify the hybrid interaction between *P. machaon* and *P. zelicaon* in this application of landscape genetics.

The extension of landscape genetics methods to understanding variation in hybridization across a landscape implies a conceptual shift in addition to the use of analyses to quantify and interpret gene flow across a geographic landscape, since gene flow between species is a very different problem from gene flow within species. In both cases, a (combined) linear scale of allele frequency is the initial biological parameter that is ultimately related to landscape variation, but in this new application to hybridization, genetic variation is first converted into a linear scale of hybridization frequency, which is then related to landscape parameters. This conversion of variation in gene flow and allele frequency allows it to be used as a measure of the penetration of genes into another kind of resistant landscape, which is the genome of hybridizing species. Such an integration of landscape variation with genomic variation in multiple interacting species (James *et al.* 2011, Hand *et al.* 2015) may ultimately provide insight into the continuing discussion of contrasting views of species: Are species units delimited by barriers to reproduction (the biological species concept), or units whose differentiation depends on an ecological template (ecological speciation; Rundle & Nosil 2005, Hendry 2009)?

Overall, this research is intended to address several facets of the interaction between evolutionary biology and systematics or taxonomy. Meaningful interpretation of species limits and hybridization dynamics requires an integrative approach using multiple data sources and data types. I explore the extent and characteristics of hybridization at multiple spatial and temporal scales in the *P. machaon* species group. This thesis adds to a growing body of research documenting the complexity of hybridization, and its potential generating biodiversity.

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Chapter 2

Multi-locus species delimitation in closely related animals and fungi: one marker is not enough

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2.1 Summary

Despite taxonomy's 250-year history, the past twenty years have borne witness to remarkable advances in technology and techniques, as well as debate. DNA barcoding has generated a substantial proportion of this debate, with its proposition that a single mitochondrial sequence will consistently identify and delimit species, replacing more evidence-rich and time-intensive methods. Although mitochondrial DNA (mtDNA) has since been the focus of voluminous discussion and case studies, little effort has been made to comprehensively evaluate its success in delimiting closely related species. We have conducted the first broadly comparative literature review addressing the efficacy of molecular markers for delimiting such species over a broad taxonomic range. By considering only closely related species, we sought to avoid confusion of success rates with those due to deeply divergent taxa. We also address whether increased population-level or geographic sampling affects delimitation success. Based on the results from 101 studies, we found that all marker groups had approximately equal success rates (~70%) in delimiting closely related species, and that the use of additional loci increased average delimitation success. We also found no relationship between increased sampling of intraspecific

variability and delimitation success. Ultimately, our results support a multi-locus integrative approach to species delimitation and taxonomy.

2.2 Introduction

Taxonomy and systematics are under renewed scrutiny and debate (e.g. Wilson 2003; Mallet & Willmott 2003; Wiens 2007; de Carvalho *et al.* 2008). The biodiversity crisis (Wilson 1992), DNA-based taxonomy (Tautz *et al.* 2003; Vogler & Monaghan 2006) versus integrative taxonomy (Will *et al.* 2005; Schlick-Steiner *et al*. 2010; Yeates *et al.* 2011), and the emergence of new methods for species delimitation (Sites & Marshall 2003; Leaché & Fujita 2010) are just a few of the issues fueling this attention. Mitochondrial DNA (mtDNA) finds itself front and center in many of these discussions. Due to its simple genetic structure, mostly uniparental inheritance and rapid rate of evolution (Avise *et al.* 1983; Moritz *et al.* 1987), mtDNA has been used extensively in species identification, delimitation, and phylogenetics for more than 20 years (e.g. Kocher *et al.* 1989; Bartlett and Davidson 1991; Folmer *et al.* 1994; Rubinoff & Holland 2005). In the last decade however, a small part of the mitochondrial genome (5' end of cytochrome *c* oxidase I, or COI gene) has been used by a variety of research groups as a universal marker, or DNA barcode, to identify most of animal life (e.g. Hebert *et al.* 2003a).

The DNA barcoding movement aims to establish a global bioidentification system, consisting of a user-friendly interface and a database of COI profiles for every animal species on the planet (Hebert *et al.* 2003a; Hajibabaei *et al.* 2007; Ratnasingham & Hebert 2007; Silva-Brandão *et al.* 2009). With this system, and advances in sequencing technology, DNA barcoding aims to identify cryptic species (Hebert *et al.* 2004; Janzen *et al.* 2005; Ball & Armstrong 2006) and unknown tissues (Wong & Hanner 2008; Waugh *et al.* 2011), associate dimorphic sexes and differing life stages (Hebert *et al.* 2004; Miller *et al.* 2005), provide an inexpensive measure of biodiversity (Smith *et al.* 2005), act to facilitate citizen science (Janzen *et al.* 2005; Savolainen *et al*. 2005), and eventually operate through a handheld DNA barcoder instrument (Janzen *et al.* 2005). Many of the early "success stories" of DNA barcoding, however, have been criticized for their phylogenetic methods and geographic limitations (Moritz & Cicero 2004; Brower 2006; Wiemers & Fiedler 2007), the use of genetic distance to assess systematic relationships (Yassin *et al*. 2010), and the suitability of mtDNA as a marker for species boundaries (Galtier 2009). Reported rates of 96-100% success in species identification (e.g. Hebert *et al.* 2003a; Barrett & Hebert 2005; Janzen *et al*. 2005) generally apply to geographically limited areas and small numbers of specimens per species (Sperling & Roe 2009; Zhang *et al*. 2010), while some studies specifically assessing DNA barcoding methodology have reported much lower success (40%: Whitworth *et al.* 2007; <70%: Meier *et al.* 2006; 77%: Elias *et al.* 2007; 80%: Meyer & Paulay 2005; 58-84%: Wiemers & Fiedler 2007).

The concept of a universal marker for accurate identification of all life holds a compelling simplicity. If its application is indeed effective at levels close to 100%, it would unquestionably be useful in many biological disciplines. Mitochondrial DNA was a logical choice for such a marker once PCR-based sequencing allowed standardized primer selection (e.g. cyt *b*: Bartlett & Davidson 1991; COI: Bogdanowicz *et al.* 1993; Sperling *et al.* 1994). However, the effectiveness of any single marker for this purpose remains open to debate, and the botanical community has since moved beyond this one marker system and is in the process of selecting a small number of markers for their molecular identification system (Hollingsworth *et al*. 2009). Despite the practice with plants, COI is increasingly being used as the primary provisional identifier of animal specimens to the species level (e.g. Ward *et al.* 2005; Burns *et al.* 2007) or to simpler entities such as molecular operational taxonomic units, or MOTU's (e.g. Hebert *et al.*2004a; Janzen *et al.* 2005). In some of these cases, there is little or no evaluation of the effectiveness of mtDNA as a diagnostic character by referring to multiple data sources.

Possible discordance between the evolutionary history of a species (i.e. species tree) and the phylogenetic reconstruction provided by a gene (i.e. gene tree) is not a new concept (Nei 1987; Pamilo & Nei 1988). Yet automated methods relying on single genes to identify species are gaining popularity (e.g. see Forister *et al.* 2008 and references within), without comprehensive testing of the efficacy of those genes. In this literature survey, we provide a taxonomically broad comparison among various classes of molecular markers in order to more comprehensively evaluate their success in delimiting closely related species, a problem that is usually only addressed case-by-case or with only a few species or markers at a time. Although systems such as DNA barcoding are concerned with a broader range of applications—e.g. utilizing a large, user-friendly database to match life stages, identify unknown tissues, etc. (see references above)—here we focus on the delimitation of closely related species, as we believe this task is of particular interest to taxonomy and systematics as sciences (de Carvalho *et al.* 2008).

To provide a more consistent basis for reevaluating the reported success of single molecular markers, we conducted a literature survey of studies that have employed multilocus species delimitation of closely related species across animals and fungi. In many studies using single locus barcoding, COI or other markers are relied on to separate both deeply divergent taxa (e.g. in different genera) as well as closely related species. However it is the closely related species distinctions that are often the most problematic, since more distantly related species can usually be more easily identified using classical morphological characters, negating the need for other methods (of course this is not always true, particularly with immature stages or partial remains). Moreover, when overall success rates are calculated, high success in delimiting different genera can mask low success in delimiting closely related species. To compensate for this limitation in documenting the effectiveness of single marker delimitation and identification, we focused solely on published comparisons that used multiple independent genetic markers to delimit closely related species, typically at the level of recently diverged sister species. We then compared the success rates of molecular marker classes for delimiting species boundaries and tested the hypothesis that increased population-level and/or geographic sampling would uncover more variation and thereby decrease species delimitation success (Moritz & Cicero 2004; Meier 2008). Finally, we addressed whether using more molecular markers increased average species delimitation success and discussed the implications of these results for the future of integrative taxonomy.

2.3 Methods

2.3.1 Multilocus literature survey

Detailed explanations of search procedures and subsequent characterization of studies for our literature survey are presented in Appendix 2.1. Briefly, we included studies published from 1990 to February 2011 that: 1) dealt with the delimitation of closely related species (generally species in the same genus); 2) compared at least two closely related but unambiguously distinct species/entities as determined by the authors; 3) sampled at least 5 specimens per species; and 4) used at least two independent molecular genetic markers (DNA or gene-based molecular markers not inherited as a single genetic block). These studies were then characterized using indices for haplotype fixation and phylogenetic congruence developed in Roe & Sperling (2007) and Roe *et al.* (2010).

Genetic markers or loci were characterized as mtDNA, ribosomal DNA (rDNA), autosomal, sex-linked, or anonymous, the last category including loci with unknown genomic locations such as microsatellites and amplified fragment length polymorphisms (AFLPs). Only nuclear-encoded rDNA genes were characterized as rDNA here. Taxa were classified as hexapods (various orders), miscellaneous (non-hexapod) invertebrates, fishes, amphibians, reptiles, birds, mammals, and fungi. Studies were sorted by clade unless sample size was low, in which case an informal paraphyletic grouping was used (e.g. invertebrates, fishes, reptiles). Plants were not examined, as the botanical community commonly uses multiple markers and similar, comprehensive analyses have already been conducted (e.g. Hollingsworth *et al*. 2009).

2.3.2 Fixation and congruence indices

Due to the heterogeneous presentation of data in diverse publications, a standardized metric was needed to quantify and compare marker success across studies. We calculated a fixation index (FI) that represented haplotype fixation and a congruence index (CGI) to describe phylogenetic correspondence for each species comparison. This approach was designed to allow standardized comparisons across taxa and studies. Although standardizing the method of analysis (for instance, reanalyzing all data with maximum likelihood) would create a more level playing field for these comparisons (though unnecessary with some datasets: Rindal & Brower, 2011), difficulties in acquiring data and the time required for such analysis made this option unviable.

The fixation index, FI, is the proportion of genetic markers whose haplotypes or alleles are reported as fixed or unique to a species (Roe and Sperling 2007). Haplotypes or alleles were classified as fixed (found only in one species) or shared (found in two or more species). We preferentially use the term haplotype to refer to both haplotypes and alleles when the distinction is unnecessary.

The congruence index, CGI, scores the phylogenetic or clustering relationship exhibited by loci, and is the proportion of fixed loci that display either reciprocal monophyly or distance-based congruence (clustering) with the species boundaries preferred by the authors of the original studies (a more detailed discussion of the effect of species concepts on these methods is found in Appendix 2.1). CGI was originally named the clustering index or CI by Roe and Sperling (2007), but we now use CGI to reduce confusion with the widely used consistency index in phylogenetics, confidence interval in statistics, and the common use of clustering to denote distance-based analyses. CGI should not be confused with I_{cong} proposed by de Vienne *et al.* (2007) for testing topological similarity between trees. CGI was scored based on the type of analysis used. For trees derived using explicitly phylogenetic methods (parsimony, maximum likelihood or Bayesian inference) loci were characterized as exhibiting either reciprocal monophyly or paraphyly/polyphyly, relative to the preferred species delimitations of authors. For trees derived using distance-based methods (e.g. neighbor-joining, UPGMA, or similar approaches), loci were scored as either congruent or incongruent with the species limits used by the authors of the studies. To avoid inflated proportions of incongruence, loci that had shared haplotypes across species (and therefore cannot form monophyletic groups or congruent clusters compared to independently determined species limits) were classified as “NA”. Thus CGI was based only on the subset of loci that had fixed haplotype differences and was a quantification of relationships among these fixed haplotypes. To summarize taxonomic subsets of the data, weighted means and 95% confidence intervals of FI and CGI were calculated following a binomial distribution (to accommodate the binomial states in the FI and CGI [Zar 1999]).

Although FI is the most easily applied measure of successful species delimitation, fixation is difficult to measure with some marker types, such as microsatellites, AFLPs, and allozymes. These markers are generally treated and reported as groups of loci in distance-based analysis, preventing the calculation of FI and reducing comparative power between marker groups. Furthermore, FI is likely to increase with the length of DNA sequenced, since longer DNA sequences are more likely to have unique mutations. Although markers analyzed using distances can only be characterized as congruent or non-congruent, this still allows calculation of CGI. Both FI and CGI are conservative in calculating success in species delimitation, since just one specimen that displays a shared haplotype or non-monophyletic relationship for that locus would cause the species to be classified as shared and paraphyletic/polyphyletic, respectively.

2.3.3 Population and geographic sampling adequacy

Our survey addressed sampling adequacy at three levels: genomic, population, and geographic. Genomic sampling was assessed by comparing different classes of genetic markers (e.g. mtDNA vs. autosomal). Population-level variation was taken into account by recording the number of specimens examined per species for every study in the literature review. Many studies sampled different numbers of specimens for different loci, and to streamline analysis in these cases we recorded the minimum number of specimens examined for all loci. To address the adequacy of geographic sampling in our literature survey, we estimated the proportion of the total geographic distribution of a species that was included in each study (see Appendix 2.1 for details). To assess the total geographic distribution (including known introductions), we preferentially used information provided by the authors of the original studies. However, if that was not sufficient we obtained this information from related literature. The total size of the species' distributions was also categorized as: A) <100 km diameter; B) 100 to 1000 km diameter; C) 1000 km to across continent, or 1000 to 5000 km for marine species; or D) more than one continent, or >5000 km for marine species. Then the extent of sampling within the total distribution was assessed in terms of 25% increments. Although these estimates are relatively coarse and dependent on the availability of knowledge about the species distributions, they provide a preliminary assessment of whether widespread or widely sampled species are more difficult to delimit using molecular markers.

2.3.4 Multilocus power analysis

A direct, although in practice substantially more complex, approach to testing whether increasing the number of loci improves species delimitation is to conduct a multilocus power analysis (see Roe *et al.* 2010). This analysis was conducted by constructing neighbor-joining trees for all individual loci, and for every combination of two, three, and four loci. For each neighbor-joining tree, congruence with the author's preferred limits for species was determined as for CGI (see above), and the average proportion of successful species delimitations (i.e. average congruence) was calculated for each number of loci. We conducted a multilocus power analysis on a subset of studies in our literature review, and details of the methodology are given in Appendix S1. We also assessed the effect of the number of loci on the proportion of successful delimitation using logistic regression. Logistic regression was conducted in R version 2.14.0 (R Development Core Team 2012) using the MASS library. Post hoc analysis was conducted using

Tukey's honestly significant differences with a Bonferroni adjustment to control for pair wise error rates.

2.4 Results

In total we examined 425 studies in detail. Of these, 324 were subsequently rejected, primarily due to low sample size. Missing data, undefined or ambiguous taxa, and inappropriate taxonomic focus, such as examinations at the level of genus or within species rather than relationships among closely related species, also contributed to many rejections (Appendix 2.3). The 101 accepted studies are summarized in Appendix 2.4, and are presented in detail in Appendix 2.5. Accepted papers examined from two to 12 closely related species and used two to 27 loci for comparison of these species.

We examined a total of 377 separately used loci across all accepted studies (Appendix 2.7). Of these, 241 showed fixed haplotypes or alleles and 108 had shared haplotypes or alleles between species (28 loci could not be classified as fixed or shared due to marker type: see Fixation and Congruence Indices, above). Reciprocal monophyly or congruence with author-defined species limits was seen in 157 loci; 111 showed either paraphyly, polyphyly, or non-congruence; and 109 were classified as "NA" (Appendix 2.7).

2.4.1 Fixation and congruence indices

Overall, the five marker classes had similar success rates in delimiting closely related species when all taxonomic groups were combined (Figure 2.1a). Autosomal loci had the lowest FI value (66% fixed versus shared haplotypes), but were surpassed only slightly by mtDNA, rDNA, and sex-linked loci (71, 74, and 74%, respectively). Mean CGI also showed a rather narrow range among loci (Figure 2.1b), with anonymous loci having the highest CGI values (76%), and autosomal loci the lowest (52%). We also examined variation in the mean FI for different taxonomic groups, since fixation acts as a general measure of delimitation success (Figure 2.2). Marker groups with less than five loci (for a particular taxonomic group) were omitted, to avoid potential sampling artifacts in mean FI and CGI values (Figure 2.1; Appendix 2.4), and as with the combined data, mean FI was highly variable for all marker classes (Figure 2.2a). Ribosomal DNA showed the smallest range (50% fixation in fungi versus 88% in miscellaneous invertebrates) and autosomal markers showed the highest (17% fixation in birds versus 82% in fungi). Hexapods were the most intensively sampled group of organisms, and were further sorted by order where sample size allowed. Generally, frequencies for FI and CGI within Hexapoda were similar to the rates for all taxa combined (Appendix 2.4). One major difference is an elevated frequency of fixed alleles in sex-linked markers (93%; Figure 2.2b), associated with increased use of these markers in the Lepidoptera and Diptera (e.g. Roe & Sperling 2007). Sex-linked markers in other groups do not show elevated fixation, although we found few studies using this marker type (one study of miscellaneous invertebrates and several of birds and mammals: Appendix 2.4). Interestingly, apart from fungi, rDNA also exhibited high FI and CGI (Figure 2.2; Appendix 2.4).

2.4.2 Population and geographic sampling analysis

Accepted studies sampled up to 320 specimens per species, but above our arbitrary cutoff of five there was a sharp decline in the number of specimens sampled per species (Figure 2.3a). No consistent relationship was present between the number of specimens sampled per species and either FI or CGI (Figure 2.3b).

Geographically, studies tended to be polarized, sampling either most of the distribution of a species or less than half of it (Figure 2.4, right Y axis). As with the number of specimens sampled per species, no relationship between FI or CGI and extent of geographic sampling was evident (Figure 2.4). When marker groups were assessed separately for both the number of specimens sampled per species and geographic sampling, no overarching trends were apparent for either FI or CGI (Appendix 2.8). When geographic sampling was subdivided by estimated global distribution an apparent trend is present toward increased FI in species with more geographically extensive ranges (Appendix 2.9). This subdivision of the data, however, contains substantial variation, and high FI values for species with more extensive geographic ranges are based on low sample sizes.

2.4.3 Multilocus power analysis

Twenty-one studies were included in this analysis, containing a total of 64 loci: Baayen *et al.* 2001, Delton Hanson *et al.* 2010, De Wit & Erséus 2010, Druzhinina *et al.* 2008, Gamble *et al.* 2008, Gangon & Turgeon 2010, Groenewald *et al.* 2005, Houston *et al.* 2010, Leaché *et al.* 2009, Lucas *et al.* 2009, Pavlova *et al.* 2008, Pérez-Losada *et al.* 2005, Puslednik *et al.* 2005, Rabosky *et al.* 2009, Reid *et al.* 2006, Roe & Sperling 2007, Roe *et al.* 2010, Rona *et al.* 2010, Thum & Harrison 2009, Welch *et al.* 2011, and Wulandari *et al.* 2009. Overall, a significantly positive relationship ($\chi^2_{0.05,4}$=20.54, P=0.0003) is observed between the average proportion of delimitation success and the number of loci included, though only modest increases are observed with sequential addition of loci (Figure 2.5).

2.5 Discussion

Using a literature review, we were able to compare species delimitation success for five classes of molecular markers across a wide range of closely related fungal and animal taxa. Three main findings were obtained from these results: 1) Used individually, all marker classes were moderately successful at delimiting closely related species; 2) increased geographic or population sampling did not significantly affect success in delimiting species; and 3) these results—particularly those of the multilocus power analysis—support investigation and use of multiple alternate markers for species delimitation.

2.5.1 Species delimitation success compared among marker classes

All marker classes showed roughly similar success rates in species delimitation when all taxonomic groups were combined (66-76% FI; Figure 2.1). Notably, mtDNA does not prove to be significantly better or worse than any other marker group. With an overall success rate of 71%, our results for mtDNA correspond well to several other estimates that were restricted to one taxonomic group (~70%: Meier *et al*. 2006; 77%: Elias *et al.* 2007). By focusing on closely related species, we intentionally distinguished success rates at this taxonomic level from surveys that include deeply divergent taxa. We feel that this focus gives a more accurate measure of delimitation success for the cases that are most in need of molecular markers – closely related species. With these limited success rates, our results emphasize that a single marker cannot consistently be used for unequivocal and universal species delimitation (e.g. Brower 2006; Meier *et al.* 2006; Elias *et al.* 2007; Roe *et al.* 2010), particularly not with confidence levels that would, for instance, hold up in a court of law (Sperling & Roe 2009). Additionally, the variability present between taxonomic groups and marker types (Figure 2.2) can be used as a guide for future investigation and development of additional universal markers for species delimitation. For example, sex-linked markers show consistently high success in delimiting closely related species in Diptera and Lepidoptera, a previously detected pattern (Diptera: Coyne & Orr 1989; Lepidoptera: Sperling 1994; Diptera & Lepidoptera: Roe & Sperling 2007).

Our multilocus power analysis indicated a significantly positive relationship between the number of loci used and species delimitation success, thus supporting previous findings using this approach (Roe *et al.* 2010). Of course, this methodology is rudimentary, and the concatenation of multiple loci with potentially different effective population sizes and evolutionary dynamics does require phylogenetic discretion. In practice, the addition of more loci is further complicated by associated costs (including both time and money), which can increase quickly and must be weighed on a project-by-project basis. Although simple, however, this analytic approach sheds light on multilocus species delimitation, and we recommend its continued use.

2.5.2 Intraspecific variation and geographic sampling adequacy

In addition to comparing the efficacy of genomically different marker classes, we investigated several sources of intraspecific variation that have been at the forefront in criticism of early DNA barcoding success stories (e.g. Moritz & Cicero 2004; Brower 2006; Zhang *et al.* 2010). Specifically, we tested hypotheses that increased population- or geographic-level sampling would decrease species delimitation success (Avise *et al.* 1987; Sperling 2003a; Moritz & Cicero 2004; Meier 2008).

Our assessment of the effects of population-level sampling used a minimum filter of at least five specimens sampled per species as a criterion for selecting studies. This gave us an ample, but not overwhelming, number of studies to work with. Of course, five specimens per species will not capture all real-world variability (DeSalle *et al.* 2005), particularly in cases with widespread species distributions (Davis & Nixon 1992; Walsh 2000). Some mtDNA barcoding proponents have proposed higher standards (10 specimens per species: Hajibabaei *et al*. 2005; 12 specimens per species: Matz & Nielsen 2005). Nonetheless, low sample size was still responsible for the highest number of rejected studies after our initial scan of the literature (154 studies: Appendix 2.6), and an additional 25 studies would have been rejected with a cut-off of 10 specimens sampled per species. Furthermore, a large number of accepted studies (40 of the 101) sampled less than 12 specimens per species (Figure 2.3a).

Contrary to theoretical expectation, we found no trend supporting the hypothesis that increasing the number of specimens sampled per species (>5) decreases FI or CGI due to increased intraspecific variation—an idea exemplified in empirical studies (e.g. Brower 2006; Meier *et al.* 2006; Segerer *et al.* 2011). The expected relationship between sampling and elevated FI or CGI may still hold if four or fewer specimens are sampled per species, but its assessment would be complicated by other factors such as the generally phylogenetic focus of such studies. We are also cautious about concluding that there is no biologically valid relationship between FI or CGI and more extensive population sampling for two main reasons. First, a review methodology relying on the literature introduces the potential for publication bias. Studies with clean, clear results are both easier to write up and easier to shepherd through review, a general phenomenon that is widely recognized (Rosenthal 1979; Csada *et al.* 1996; Johnson & Dickersin 2007; Lehrer 2010). Second, the occurrence of selective sweeps not only within species, but also introgression between species, is becoming more apparent (see Chan & Levin 2005 and references within). Either, or both, of these issues could confound our assessment of intraspecific variation, and identifying the exact cause is beyond the scope of this study.

Inadequate geographic sampling is another common critique of studies using mtDNA for species delimitation (Sperling 2003b; Will & Rubinoff 2004; Moritz & Cicero 2004; Brower 2006). Empirically, inter-population differentiation has been shown to be a large contributor to genetic variance (Ward & Grewe 1994; Ramachandran *et al*. 2005; Lukhtanov *et al.* 2009; Bergsten *et al.* 2012), although we saw no relationship between increased geographic sampling and FI or CGI, even when species were subdivided by their estimated global distributions. As with sample sizes, this finding may reflect publication biases. Approximately 40% of the studies were not concerned with extensive geographic sampling. Many focused on hybrid zones and/or introgression (e.g. Berthier *et al.* 2006; Bull *et al.* 2006; Gompert *et al*. 2006; Vogel & Johnson 2008), small areas of geographic overlap for phylogeographic analysis (e.g. Newbound *et al.* 2008; Yannic *et al.* 2010; Schoville *et al.* 2011), or single or few localities for other purposes (e.g. testing the efficacy of DNA barcoding methodology: Elias *et al.* 2007; estimating divergence times: Rona *et al.* 2010). Consequently we are unsure of the biological validity of these results, and also cannot discount the possibility of publication bias or selective sweeps/introgression, as discussed above.

Interestingly, several recent DNA barcoding studies have also addressed the issue of geographic sampling, with differing results; while Lukhtanov *et al.* (2009) found substantially increased intraspecific variability with increased geographic sampling, Hebert *et al*. (2010) did not. Both of these studies, however, were limited to one taxonomic group, and were further distanced from our results by the inclusion of numerous deeply-divergent species. As other empirical studies continue to reinforce the importance of capturing interspecific variability for species delimitation (Brower 2006; Meier *et al.* 2006; Segerer *et al.* 2011), it is clear that summarizing these effects requires more work.

2.5.3 mtDNA, species delimitation, and taxonomy

The third, and we believe most important, issue raised by our results concerns the fundamental nature of species delimitation as a taxonomic approach. Although DNA barcoding is useful in many applications, limitations in methodology and the nature of mitochondrial evolution decrease its applicability for detailed systematic or taxonomic analysis, particularly for closely related species (DeSalle *et al*. 2005; Will *et al.* 2005; de Carvalho *et al*. 2008). COI—or any other molecular marker for that matter—serves only as a rough guide for successfully delimiting species. Although some groups of organisms are well delimited by a single marker, many will not fit into this single-locus conceptual construct. Species may be considered to be hypothetical vessels to hold and characterize variation, and as hypotheses, are either supported or rejected by data (De Queiroz 2007; Padial & de La Riva 2010; Yeates *et al.* 2011). Despite recent discussions addressing contrasting goals and definitions of DNA barcoding, taxonomy, and systematics (e.g. Vogler & Monaghan 2006; DeSalle 2007; Waugh 2007; Brower 2010; Ebach 2011; Stevens *et al.* 2011), each of these fields is concerned with species as taxonomic hypotheses. By limiting the amount of genomic variation (i.e. using only one marker), or intraspecific variation that is sampled (as discussed above), we limit the ability to effectively realize patterns and formulate alternative hypotheses concerning species boundaries.

Ultimately, a balance must be met between the standardization and automation advocated by DNA barcoding, and the systematic and taxonomic view of a species as a hypothesis. Therefore, we argue in favor of standardization of multiple markers within groups of animals (e.g. van Nieukerken *et al.* 2012), a task that our taxonomically partitioned results can assist, and iterative or integrative approaches to species delimitation and taxonomy (see Yeates *et al.* 2011). The importance and the added complexity of incorporating multiple lines of evidence in species delimitation are not new concepts (e.g. Wilson & Brown 1953). Reliance on multiple molecular markers may lead to more cases of incongruence, as compared to a "barcode species concept" (Rubinoff 2006), but the aim in this endeavor is the delimitation of evolutionary significant units rather than self-referential consistency. Furthermore, detection of incongruence leads to greater evolutionary understanding of phenomena such as introgression, population structure, and sex-biased gene flow (Funk & Omland 2003; Rubinoff & Holland 2005; Marko & Hart 2011). Power analyses evaluating the need for multiple markers are available for plants (e.g. Hollingsworth *et al.* 2009; Burgess *et al.* 2011), and we have attempted to move in this direction for animals and fungi (e.g. Roe *et al.* 2010); however there is a clear need for further studies of this kind. Ultimately, by capturing as much natural variation as possible within biologically meaningful species limits, our knowledge of those species units will have more universal applications in the ways that matter to us all.

2.6 Conclusions

This is the first taxonomically comprehensive review of the efficacy of different marker groups for the delimitation of closely related species. Through the use of strict screening methods we have shown that all marker groups have relatively equal success in delineating closely related species, and that using more markers increases average delimitation success. Unexpectedly, we found no relationship between population-level or geographic sampling and delimitation success, although this may be an artifact of our review methodology and deserves more rigorous and systematic investigation. Ultimately, we support a hypothesis-based, integrative approach to species delimitation. Divorcing our knowledge of real biological complexity from the operational process of species delimitation would only serve to confine our knowledge of biodiversity, and suspend progress in taxonomy, systematics, and biology as a whole.

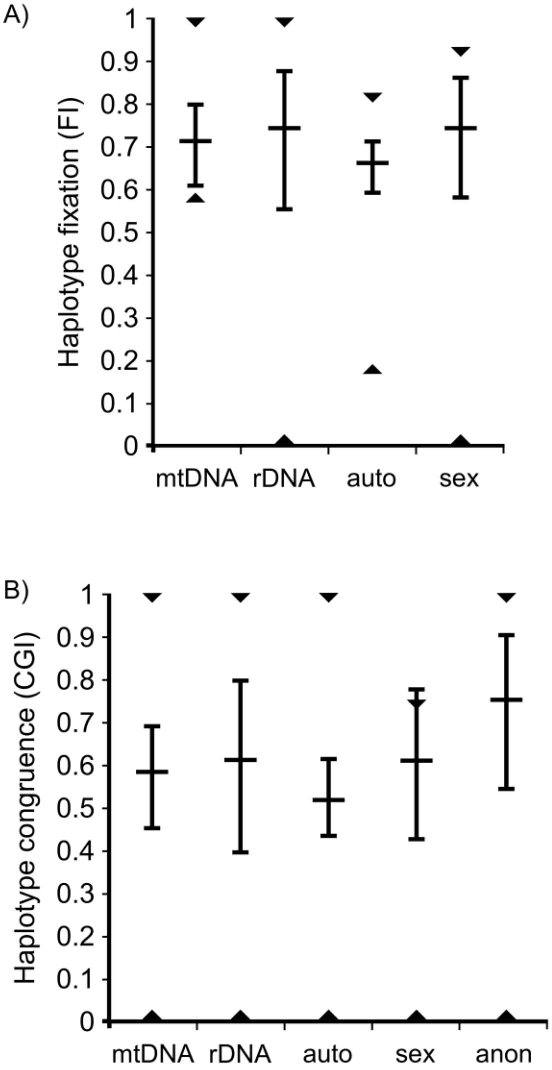

Figure 2.1 Haplotype fixation index (A) and congruence index (B) subdivided by marker classes. Central horizontal lines represent mean FI or CGI for all loci, and vertical bars represent 95% confidence intervals. Triangles represent minimum and maximum FI or CGI values when means are partitioned by taxonomic group. auto: autosomal; sex: sex-linked; anon: anonymous.

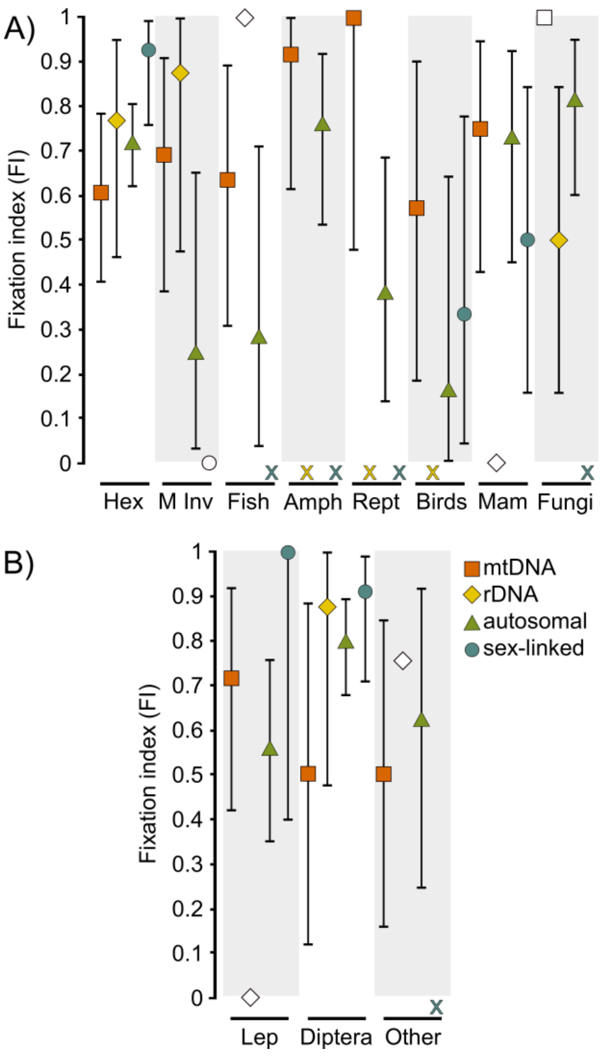

Figure 2.2 Haplotype fixation indices subdivided by taxonomic group. 2A) All major organism groups; 2B) Hexapoda further divided by order. Error bars correspond to upper and lower 95% confidence intervals. Empty symbols with no error bars correspond to mean FI of marker groups with <5 loci sampled. Colored X's correspond to marker groups with no data available. "Other" in Figure 2.2b includes orders Hemiptera, Hymenoptera, and Odonata. Fish: fishes; Hex: hexapods; M Inv: miscellaneous invertebrates; Amph: amphibians; Rept: reptiles; Mam: mammals; Lep: Lepidoptera.

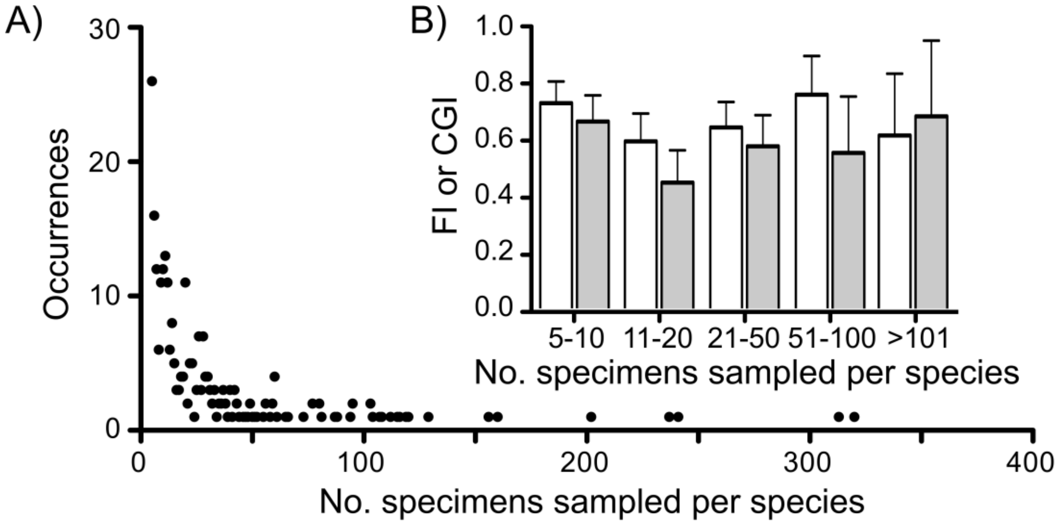

Figure 2.3 Sampling adequacy relative to the number of specimens sampled per species. A) Number of specimens sampled per species for every species considered in literature review (n=271 species). When a different number of specimens per species was sampled for different loci, the count recorded is the minimum number of specimens sampled for all loci. B) Fixation (white columns) and congruence (grey columns) indices grouped by number of specimens sampled per species, with 95% confidence intervals (FI: n=271 species; CGI: n=227 species).

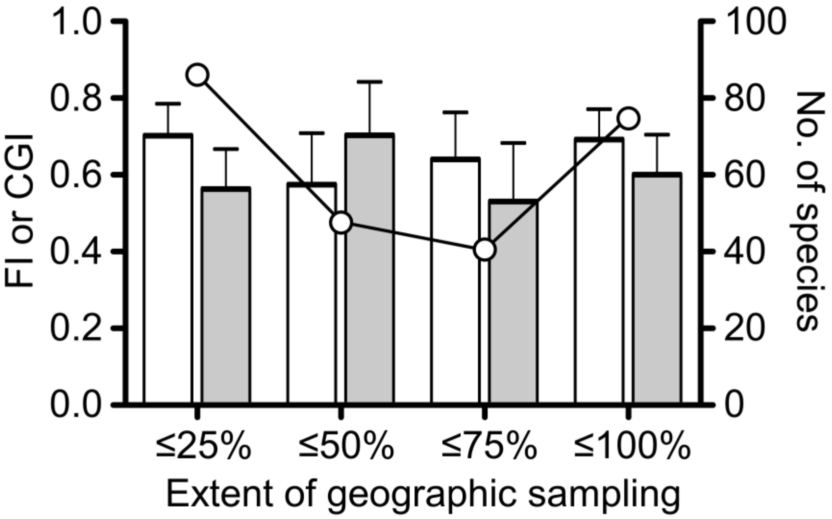

Figure 2.4 Adequacy of geographic sampling. Columns and 95% confidence intervals correspond to fixation (white columns: n=246 species) and congruence (grey columns: n=205 species) indices within each geographic sampling category (left Y axis). Open circles and connecting line represent the number of species sampled in each category (right Y axis).

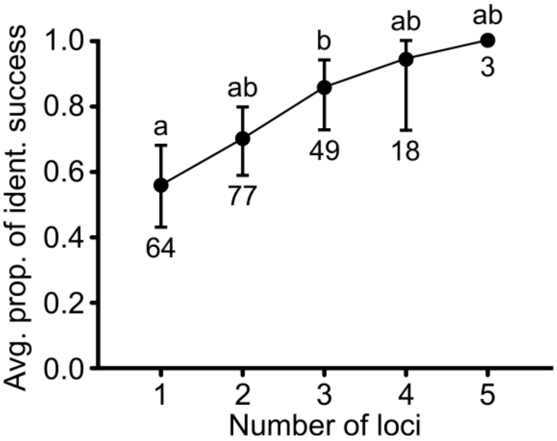

Figure 2.5 Results of the multilocus power analysis. Black circles represent the average proportion of identification success for each number of loci, and error bars represent 95% confidence intervals. Numbers below each circle/error bar indicate the sample size for each category. Data points with different letters indicate significant differences in identification success (α=0.05).

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Chapter 3

Repeated reticulate evolution in North American *Papilio machaon* group swallowtail butterflies

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3.1 Summary

Hybridization between distinct populations or species is increasingly recognized as an important process for generating biodiversity. However, the interaction between hybridization and speciation is complex, and the diverse evolutionary outcomes of hybridization are difficult to differentiate. Here we characterize potential hybridization in a species group of swallowtail butterflies using microsatellites, DNA sequences and morphology, and assess whether adaptive introgression or homoploid hybrid speciation was the primary process leading to each putative hybrid lineage. Four geographically separated hybrid populations were identified in the *Papilio machaon* species group. One distinct mitochondrial DNA clade from *P. machaon* was fixed in three hybrid taxa (*P. brevicauda*, *P. joanae*, and *P. m. kahli* Chermock & Chermock, 1937), while one hybrid swarm (*P. zelicaon* x *machaon*) exhibited this hybrid mtDNA clade as well as widespread parental mtDNA haplotypes from both parental species. Microsatellite markers and morphology showed variable admixture and intermediacy, ranging from signatures of prolonged differential introgression from the paternal species (*P. polyxenes*/*P. zelicaon*) to current gene flow with both parental species. Divergences of the hybrid lineages dated to early- to mid-

Pleistocene, suggesting that repeated glaciations and subsequent range shifts of parental species, particularly *P. machaon hudsonianus* Clark, 1932, facilitated initial hybridization. Although each lineage is distinct, *P. joanae* is the only taxon with sufficient evidence (ecological separation from parental species) to define it as a homoploid hybrid species. The repetition of hybridization in this group provides a valuable foundation for future research on hybridization, and these results emphasize the potential for hybridization to drive speciation in diverse ways.

3.2 Introduction

Hybridization between distinct populations or species has historically been considered trivial in the overall diversification of animal life (e.g. Wagner 1970) or as a countervailing force to speciation (Abbott *et al.* 2013). Modern molecular methods have turned this notion on its head, and it is becoming increasingly clear that hybridization is both more prevalent and evolutionarily more important than previously thought (Mallet 2007, Nolte & Tautz 2010, Butlin *et al.* 2012, Harrison 2012, Abbott *et al.* 2013). Hybridization can have a continuum of complex outcomes in speciation. It can inhibit speciation by homogenizing distinct populations through neutral diffusion (Hewitt 1988), referred to as “breakdown” of reproductive isolation (Taylor *et al.* 2006, Harrison 2012). When selection against hybrids limits neutral introgression between hybridizing populations, tension zones can form (Barton & Hewitt 1985), with variable outcomes depending on the strength of selection. If selection is weak, tension zones can stabilize, thereby stalling speciation while maintaining genetic differentiation (e.g. Ruegg 2008). If selection against hybrids is strong, reduced hybrid fitness can reinforce premating barriers (e.g. assortative mating), strengthening barriers to gene exchange and advancing speciation (“reinforcement”: Servedio & Noor 2003, Via 2009). Alternately, genetic differentiation can increase through adaptive introgression from one species to another (Hedrick 2013), or unique admixture of new, hybrid species (Rieseberg *et al.* 2003, Mallet 2007, Jiggins *et al*. 2008). These phenomena are not mutually exclusive, and variation and combinations exist along the continuum. Ultimately, our interpretation of hybridization only captures "a single snapshot of a complex and continuously changing interaction" (Abbott *et al.* 2013).

Homoploid hybrid speciation (hereafter referred to as hybrid speciation) and adaptive introgression are particularly important promoters of differentiation, as they can generate novel hybrid entities as well as the resources to fuel adaptive divergence of preexisting species (Butlin *et al.* 2012, Abbott *et al.* 2013, Hedrick *et al.* 2013). Although both processes involve hybridization of distinct populations or species, hybrid speciation culminates with adaptive novel hybrid combinations forming a distinct and independent hybrid taxon (Gross & Rieseberg 2005, Mallet 2007, Dasmahapatra *et al.* 2012). In adaptive introgression, recombinant hybridization passes adaptive variation from one species to another, replacing less adaptive portions of the original genome, but maintaining the majority of that original genome (Whitney *et al.* 2010, Pardo-Diaz *et al.* 2012, Hedrick 2013). Defining hybrid speciation thus necessitates identification of novel, hybrid traits or combinations that allow hybrids to be distinguished from parental taxa, regardless of the level of genetic admixture (Abbott *et al*. 2013). Identification of these traits can be difficult both theoretically and empirically, and is further complicated in systems exhibiting complex phylogenetic histories (McGuire *et al.* 2007, Melo-Ferreira *et al.* 2012), differential influences of parental taxa (Jiggins *et al.* 2008), and ancient or repeated hybridization (Genner & Turner 2012). However, complex systems that exhibit repeated hybridization at different temporal and spatial scales provide useful "natural laboratories" for understanding the ramifications of hybridization and downstream processes at an evolutionary scale (Hewitt 1988, Buggs 2007).

Swallowtail butterflies of the *Papilio machaon* species group (Lepidoptera: Papilionidae: the Old World swallowtails) provide a diverse model system in which to study hybridization. Species limits and systematic relationships have been notoriously difficult to resolve (e.g. Ae 1979, Clarke & Larsen 1986) in part due to a plethora of ecological races, color morphs, and incomplete reproductive barriers, as well as abundant natural hybridization (Sperling 1987, Sperling 1990, Sperling & Harrison 1994). Additionally, the genus as a whole has been influential in the development of many prominent theories in biology (speciation: Mallet 2004, the biological species concept: Mayr 1955, coevolution: Ehrlich & Raven 1964, mimicry: Brower 1958, etc.), and has had a disproportionate influence on our understanding of the genetic and ecological dynamics of hybrid speciation (Scriber & Ording 2005, Kunte *et al.* 2011, Zhang *et al.* 2013). In North America, six species are currently recognized within the *P. machaon* species complex: *Papilio brevicauda, P. indra, P. joanae, P. machaon* (the only member to have a Holarctic distribution, Sperling 1987), *P. polyxenes*, and *P. zelicaon* (Pelham 2008). *Papilio indra* is the only North American member of the species group to consistently have distinctive genitalia and adult wing pattern (Sperling & Harrison 1994) and multiple genetic studies place it as the sister species to the rest of the clade (Emmel & Emmel 1964, Condamine *et al.* 2012). The five remaining North American species share many wing pattern characteristics, but can generally be separated into yellow- or black-morph species (Figure 3.1), although color polymorphism is widespread (e.g. Chermock & Chermock 1937, Remington 1968; also Sperling 1987). Larval hostplant use in the *P. machaon* group is confined to species of Asteraceae, Apiaceae (Umbelliferae), and Rutaceae, and while geographical specialization is the norm, uncommon species/hostplant pairings have been widely observed, suggesting that ecological differentiation is not strongly tied to larval hostplant constraints (Sperling 1987, Sperling 1990).

The impetus for the research reported here was the finding that *P. brevicauda* and *P. joanae*, although morphologically quite similar to *P. polyxenes* (Figure 3.1), share mitochondrial DNA (mtDNA) restriction-site patterns with *P. machaon* (Sperling & Harrison 1994). Both *P. brevicauda* and *P. joanae* are geographically separated from *P. machaon*. *Papilio brevicauda* is restricted to the Maritime Provinces of Canada, often frequenting bluffs near the sea (Morris 1980, Layberry *et al.* 1998). *Papilio joanae*, on the other hand, is an endemic species of closed woodland habitats of the Ozark plateau in Missouri (Scott 1986, Allen 1990), and because of its restricted range has been classified as "vulnerable" by conservation associations (Schweitzer *et al.* 2011). Both of these taxa have variably been considered species in good standing (*P. brevicauda*: Clarke & Sheppard 1955; *P. joanae*: Pelham 2008) or subspecies/races of *P. polyxenes* due to their morphological resemblance (*P. brevicauda*: Rothschild & Jordan 1906; *P. joanae*: Scott 1986). More recently, however, they have been described as close relatives of *P. machaon* based on the mtDNA findings of Sperling & Harrison (1994) (Tyler *et al.* 1994, Layberry *et al*. 1998, Pelham 2008). Mitochondrial DNA haplotypes identical to those in *P. brevicauda* and *P. joanae* were also found by Sperling and Harrison (1994) in putative hybrids in southwestern Alberta (*P. machaon* x *P. zelicaon*: Sperling 1987) and southern Manitoba (*P. machaon kahli*: Chermock & Chermock 1937, or *P. machaon* x *P. polyxenes* after Sperling 1987). Both of these populations represent morphological intermediates between the putative parental taxa, and while morphology and allozymes have been studied in the SW Alberta populations (Sperling 1987, Bird *et al.* 1995), those in southern Manitoba have received less attention. Following Sperling (1987) and Pelham (2008), respectively, we refer to the populations in southwestern Alberta as *P. zelicaon* x *machaon*, and those in southern Manitoba as *P. machaon kahli*. “Parental taxa” refer to *P. machaon*, *P. polyxenes*, and *P. zelicaon*.

Despite the discordant mtDNA affinities of these taxa and their recognition as close relatives of *P. machaon*, no research has followed up on the hypothesis of ancient hybridization set out by Sperling & Harrison (1994). Here we evaluate this putative hybridization across North America using mitochondrial and nuclear DNA sequences, microsatellite markers, molecular dating, morphological analysis of wing pattern, and assessment of ecological characteristics. Given the apparent evolutionary complexity of the group, we approach this evaluation using the preexisting taxonomic entities most prevalent in the literature (*sensu* Sperling 1987, Pelham 2008) to guide our interpretation of these lineages, and ask to what extent each putative hybrid lineage (*P. brevicauda*, *P. joanae*, *P. m. kahli*, and *P. zelicaon* x *machaon*) exhibits characteristics of the putative parental species (*P. machaon*, *P. polyxenes*, and *P. zelicaon*). Using these data, we then evaluate whether significant support exists to classify each of these lineages as hybrid species, or if they appear to be the result of adaptive introgression. Following Abbott *et al.* (2013), we consider a hybrid species to be one that demonstrates novel characteristics or combinations that distinguish, and ideally, reproductively isolate the hybrid from *both* of its parents. Specifically, we use our genetic and morphological data to assess the genealogical discordance and distinctness of hybrid lineages, and focus on ecological separation to guide our inference of reproductive isolation (e.g. host plant, habitat, flight period, etc.). Genealogical and morphological distinctness, and reproductive isolation are considered to constitute significant evidence for the hypothesis of hybrid speciation.

3.3 Methods

3.3.1 Specimen selection and DNA extraction

Specimens were selected to represent the taxonomic breadth and diversity of the *P. machaon* species group in North America, as well as by the availability of DNA and wing vouchers. Species designations followed work on the same specimens or populations included in prior studies (e.g. Sperling 1987, Sperling & Harrison 1994). Morphology, geography, and in some cases larval host plant information were used to identify new specimens to species. We define species, in accordance with the genomic integrity species definition (Sperling 2003), as populations able to maintain their genomic integrity upon contact with each other, or allopatric populations with genetic divergence proportional to that found in closely related sister species. Slightly different subsets of specimens were used for each analysis due to DNA/wing quality limitations and voucher availability. Details are described below and species-specific sample sizes for each analysis are given in Table 3.1. All specimens used in this study, as well as associated locality and voucher deposition information, are given in Appendix 3.1 (some geographic coordinates for private land and historic samples are not displayed). Collecting of recent material in provincial parks in Alberta and British Columbia was conducted under permit numbers 10-097 and 105180, respectively, and collecting on private land was done with the owner's permission. All freshly collected material was collected with an aerial net (for adults), or hand collected as larvae and reared to the adult stage, then killed and stored at -70°C.

Sequence data generated by Caterino & Sperling (1999), Reed & Sperling (1999), Zakharov *et al.* (2004), and Bromilow & Sperling (2011) was retrieved from GenBank for a number of specimens within the *P. machaon* species group (Table 3.1), as well as *P. xuthus* Linnaeus, 1767, which served as an outgroup in the phylogenetic analyses (Zakharov *et al.*

2004). Data retrieved from these sources varied from the full COI/COII genes (including the intervening *tRNA-leucine* gene; 2288 bp) to partial COI (394 bp), and the full EF-1α gene (1010 bp). Additional mitochondrial sequence data was generated for this study from pre-existing DNA extractions representing the major mtDNA restriction-site haplotypes found in Sperling & Harrison (1994). More recently collected butterflies were also sequenced for COI, and in these cases, adult butterflies (field collected or reared from field-collected larvae) were killed and stored at -70°C. Additional EF-1α data was also generated for a subset of specimens from each species, although due to the limited and discordant phylogenetic information content of this gene, sequencing was not pursued for the remainder of the specimens (see Results). Microsatellite analysis was attempted on all specimens for which sequence data was available, except for a handful of specimens for which no DNA remained (including the only available specimen of *P. hospiton*). All recent DNA extractions (for both sequence data and microsatellites) were carried out using Qiagen DNeasy® Blood & Tissue extraction kits (QIAGEN, Mississauga, Ontario, CAN) using leg or thoracic tissue.

3.3.2 Sequence data

Polymerase chain reactions (PCRs) were conducted in 50 μL reactions with a Biometra TGradient thermal cycler (Biometra, Goettingen, DE), including the following reagents: for COI/COII, 5 μL 10x PCR buffer (Promega, Madison, WI, USA), 3 μL of 25 mmoles/μL MgCl2 (Promega), 1 μL of 10 mmoles/μL dNTPs (Roche, Switzerland), 2 μL of each forward and reverse primer in 5 pmol/μL concentrations, 1 μL of 5 U/μL *Taq* polymerase (QIAGEN), 1 μL DNA, and 35.5 μL autoclaved Millipore water; for EF-1α, all reagent quantities were identical except for 2 μL of MgCl2 and 36.75 μL Millipore water. Reactions were conducted with a hot start (introducing *Taq* Polymerase after the initial 2 minute, 94°C denaturation period) followed by 35 cycles of 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute for COI/COII, and 35 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 1.5 minute for EF-1α. All reactions were finished with a 7-minute final extension at 72°C. Primers used in this study are given in Appendix 3.3. PCR purification was conducted with either a Qiagen QIAquick® PCR purification kit or a Qiagen QIAEX II® agarose gel extraction kit (QIAGEN). Sequencing reactions were carried out in both directions using a DYEnamic™ ET terminator cycle sequencing kit (Amersham Pharmacia Botech, Cleveland, Ohio, USA), and either filtered through Sephadex-packed columns or ethanol precipitated before being dried, resuspended, and fractionated on either an ABI PRISM® 377 or 3730 automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Sequences were aligned using Mesquite v2.75 (Maddison & Maddison 2011) and ClustalW v2.0.12 (Larkin *et al.* 2007) using default settings. Alignment quality was checked by eye, but major adjustments were not necessary due to the absence of indels and introns. For the EF-1α sequences, double peaks consistently observed in the electropherograms were assumed to be the result of heterozygotes, and were coded using IUPAC ambiguity codes. The ends of sequences were trimmed to facilitate collapsing strictly redundant haplotypes in MacClade v4.08a (Maddison & Maddison, 2005). Although in some cases this removed variable characters from the matrix for COI/COII, overall topological patterns were not affected.

3.3.3 Phylogenetic analyses

Phylogenetic analyses were conducted using multiple optimality criteria to ensure that the choice of analytical method did not bias conclusions. Unweighted, unordered MP (Fitch, 1971) searches were conducted in PAUP* 4.0b10-x8 (Swofford, 2002), with heuristic strategy of 1000 replicates of random sequence addition (holding 10 trees per replication), tree-bisection and reconnection branch swapping (TBR; Swofford & Olsen, 1990) and no limit to the maximum number of trees retained per replication. Bootstrapping (Felsenstein, 1985) with heuristic strategy of 100 replicates of random sequence addition (holding 100 trees per replication), TBR, and a maximum number of 100 trees (of minimum score 1) retained per replication, was conducted to test node support. To test for incongruence between data sets, Templeton (1983) tests of data heterogeneity were conducted in PAUP* on a subset of taxa (specimens that had data for both COI/COII and EF1α) to identify if one data set could statistically reject the topology of the tree given by the other data set. In these tests, 50% majority rule consensus trees of the individual complete data sets (from maximum parsimony searches) were constrained to the alternative data set and evaluated using the PSCORES command in PAUP*.

Maximum likelihood analyses were conducted in GARLI v0.951-GUI (Zwickl, 2006) applying models of evolution as predicted by the Akaike Information Criterion (Akaike, 1974) in jModelTest 2.1.1 (Darriba *et al.* 2012, Guindon and Gascuel, 2003). The following models of evolution were used: extended COI/COII and complete EF-1α: Transition model (TIM) + I; complete COI/COII and extended EF-1α: TIM + Γ. All parameter values were specified in GARLI, and 100 bootstrap replicates were conducted.

Bayesian inference was conducted in MrBayes v3.2 (Ronquist *et al.* 2011). Transition models of evolution are not applicable in MrBayes so were simplified, as in Zakharov *et al.* (2009), as follows for these analyses: extended COI/COII: general time reversal (GTR; Tavare, 1986) + I + Γ; complete COI/COII and extended EF-1α: GTR + Γ; and complete EF-1α: Hasegawa, Kishino, and Yano model (HKY; Hasegawa *et al.* 1985) + I. Two million generations were run with trees being sampled from both runs every 100 generations, and default chain settings/temperatures. No priors were specified. The average standard deviation of split frequencies was observed during the run and the potential scale reduction factors were observed after the run to ensure that independent simulations were converging (values should approach zero and one, respectively). Burn-in trees were estimated visually for both runs in the log-likelihood overlay plot, and 25% of sampled trees were removed for burn-in. Posterior probability (clade credibility) values were calculated in MrBayes, and a 50% majority rule consensus tree was constructed in PAUP* after removing burn-in trees.

3.3.4 Microsatellite markers

Ten of 17 microsatellite loci developed by Zakharov & Hellman (2007) were reliably amplified in all species of interest (Appendix 3.2). Reverse primers for six of these loci were "PIGtailed" to decrease non-template nucleotide addition that hinders genotyping (Brownstein *et al*. 1996). Microsatellite amplification was conducted using universal fluorescently labeled M13 forward primers (Schuelke 2000), and sequence-specific primers mixed in a ratio of 4:1 reverse primer: M13 tailed forward primer. PCR reactions were conducted in 15 μL volumes containing 1.5 μL 10x microsatellite PCR buffer, 1.5 μL of 25 mM $MgCl_2$, 0.3 μL dNTPs, 0.48 μL 4:1 sequence specific primer mix, 0.48 μL universal fluorescent-labeled M13 primer, 0.2 μL *Taq* DNA polymerase (Pickard Laboratory, University of Alberta), and 2.5 μL DNA, under the following cycling conditions: 10 min at 94°C, 38 cycles of [30 sec at 94°C, 45 sec at 56°C or 57°C (Appendix 3.2)], and 45 sec of 72°C, and followed by a final extension of 10 min at 72°C. Amplified fragments were run on an ABI Prism 3730 Analyzer (ABI), with a Genescan® LIZ-500 size standard, and genotyped using Genemapper® v4.0 (ABI). Descriptive statistics and measures of population differentiation (F_{ST}) were calculated using GENODIVE (Meirmans & Van Tienderen 2004).

3.3.5 Individual-based clustering

Bayesian clustering of individual microsatellite data was conducted in STRUCTURE (Pritchard *et al.* 2000) using an admixture model and independent allele frequencies for all analyses. A burn-in period of 150,000 Markov chain Monte Carlo (MCMC) generations was followed by 500,000 generations for $k = 1$ through $k = 10$, with 10 iterations for each value of k. The most likely number of genetic clusters was calculated by evaluating the likelihood of the data (lnP(D| K); Pritchard *et al.* 2000) and Δk (Evanno *et al.* 2005) with the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). CLUMPP v1.1.2 (Jakobson & Rosenberg, 2007) was used to average replicate runs for each k value. STRUCTURE was also run using putative parental taxa as training sets, species determinations as “population” priors, and on a dataset including *P. indra*, the outgroup. Results using either training sets or population priors did not differ from analyses without these conditions, so the latter are presented here. Analyses including *P. indra* are provided in Appendix 3.6. Sub-structure was assessed in the overall analysis (by calculating ancestry for suboptimal Δk values), and by breaking the dataset up according to the overall $k = 2$ results where individuals with ≥ 70% *machaon*-like ancestry were treated separately from remaining individuals. These methods produced similar assessments of substructure: The latter are focused on in the results section, as they provided clearer demarcation of substructure, and the former are provided in Appendix 3.7.

To investigate relatedness between clusters, we also conducted discriminant analysis of principal components (DAPC: Jombart *et al.* 2010), which submits genetic data to a principal component analysis (PCA) before conducting discriminant analysis (DA) on those principal components. This multivariate discriminant method does not attempt to minimize Hardy-Weinberg and gametic equilibrium (as does STRUCTURE, Pritchard *et al.* 2000), and is therefore potentially more suited to this style of phylogenetically-oriented sampling. In maximizing between- and minimizing within-group variability (Jombart *et al.* 2010), DAPC has also been shown to be more powerful and accurate with hierarchical relationships (Kanno *et al.* 2011), which might be predicted in situations of hybridization and differential introgression. We implemented DAPC in R v3.0.1 (R Development Core Team, 2013) using *adegenet* v1.3.1 (Jombart, 2008). To provide comparison with STRUCTURE, the *find.clusters* function was used with default parameters, retaining all principal components (PCs), to find the ideal *k* value.

To visualize relationships between clusters using DAPC, the *optim.a.score* function was used to determine the optimal number of PCs to retain in the DA. In this function, 25 full simulations (parameter *smart*=FALSE) of a preliminary DAPC run (retaining the number of PCs corresponding to one-third the sample size of the run) are reiterated by the *optim.a.score* function to determine the ideal number of PCs to retain. This optimal number of PCs was then used in the final DAPC. All discriminant functions were retained for all DAPCs. The *xvalDapc* function was also used as an alternative to *optim.a.score*, and presented consistently similar, though somewhat larger (approximately 10-15 additional PCs), values of the optimal number of PCs. Due to uneven sample sizes between clusters, and potential biases in the *xvalDapc* function in these cases, *optim.a.score*'s determination of the ideal number of PCs was used (though retaining *xvalDapc*'s ideal number of PCs did not change the overall clustering pattern). Additionally, *optim.a.score*'s smaller optimal number of PCs graphically clustered the groups with smaller samples sizes closer to the main groupings, allowing better determination of relationships. DAPC was also conducted on a dataset including *P. indra*, the outgroup, and those results are provided in Appendix 3.8.

3.3.6 Morphometrics

Six wing morphometric characters were used as in Sperling (1987 pg 208-209): A) extent of yellow scaling in cell Cu2, in anal margin of dorsal hindwing, B) shape of pupil in anal eyespot of dorsal hindwing, C) extent of black scales between blue and red portions of anal eyespot of dorsal hindwing, E) extent of yellow scales in basal half of disc of ventral forewing, F) extent of yellow scales of postmedian yellow band in apical cell of ventral forewing, and G) number of cells with orange patch in postmedian area of ventral hindwing, plus one. Right wings were used, unless characters were only visible on left wings. The quality and preserved tissues of 20-30 year old voucher specimens limited the amount of useable characters to those on the wing, and wing length characters were ignored due to large amounts of missing data associated with worn/tattered specimens; some specimens for which DNA-based data were available were too damaged to be scored (Table 3.1). Multiple correspondence analysis (MCA), an alternative to PCA for categorical variables, was conducted in R v3.0.1 (R Development Core Team, 2013) with the package FactoMineR (Lê *et al*. 2008, Husson *et al*. 2012).

3.3.7 Molecular dating

Molecular dating of the complete COI/COII data was implemented in BEAST v1.6.2 (Drummond & Rambaut 2007), using BEAUti to generate the associated *xml* input file. Due to the paucity of fossil papilionids (see Condamine *et al.* 2012), secondary calibrations from previous molecular dating studies in the Papilionidae were used to calibrate the tree. Five calibration points (shown in Appendix 3.5), including the root calibration, were used from the soft-bound age estimates calculated by Condamine *et al*. (2012) (nodes 167, 168, 169, 171, and 172). These were applied using the *tmrca* prior with a uniform distribution. Additionally the *Site Model* prior was set to match that used for BI (GTR + Γ), and the *Clock Model* prior was estimated with a relaxed, uncorrelated lognormal clock (Drummond *et al.* 2006). Both the Yule (Yule 1924) and Birth-Death (Gernhard 2008) process speciation *Tree Priors* were used, but did not change the results appreciably; results from the Birth-Death process prior will be reported. Five independent runs of 100×10^6 generations, being sampled every 1×10^3 generations, were run and combined using LOGCOMBINER. To ensure proper parameter estimates, TRACER was used to check effective sample sizes. A burn-in of 25% of the trees from each run was removed before all combined trees were summarized in TREEANNOTATOR.

3.4 Results

3.4.1 DNA sequence properties

Sequence data was collected from 133 individuals representing 22 taxa (Table 3.1, GenBank accession numbers: Appendix 3.1). DNA alignments for the gene regions *cytochrome oxidase I/II* (COI/COII) and *elongation factor-1α* (EF-1α) were 2288 bp and 1010 bp respectively (Table 3.2), although most sequences were shorter than this total. To test whether missing data affected overall topology, all phylogenetic analyses were conducted with the “extended” dataset (including missing data) and with a “complete” dataset that included only shorter sequence regions present in all specimens. The shorter alignments consisted of 306 and 418 bp, for COI/COII and EF-1α respectively.

The COI/COII data included 133 specimens that were consolidated to 54 unique haplotypes in the complete dataset, and the EF-1α data included 27 specimens that contained 25 unique genotypes (Table 3.1, Appendix 3.1). Tests of topological incongruence concluded that the two data sets (COI/COII and EF-1α) were not homogenous. Constraining the abridged COI/COII data to the EF-1α topology supported incongruence between the datasets (p=0.0002); reversing that constraint (i.e. constraining EF-1α data to COI/COII topology) did not (p=0.0588), as could be expected since the EF-1α data contained relatively little variation, and hence phylogenetic information (Table 3.2), and had few resolved clades (see below).

3.4.2 Phylogenetic relationships

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses produced very similar, although not identical, topologies for each data set. Measures of branch support (MP and ML bootstrap and BI posterior probability) generally increased for phylogenies based on extended data for COI/COII, but decreased for EF-1α. Overall topology was not affected by the inclusion of regions of missing data. Bayesian 50% majority rule consensus trees are shown here for the extended datasets (Figure 3.2), and summary information for MP and ML are presented in Table 3.2.

COI/COII supported previously resolved relationships (Sperling 1987, Caterino & Sperling 1999, Zakharov *et al.* 2004, Simonsen *et al.* 2011, Condamine *et al.* 2012) between *P. machaon*, *P. hospiton* Gené, 1839, *P. polyxenes*, and *P. zelicaon* (Figure 3.2b). The mitochondrial phylogeny failed to separate subspecies within *P. machaon* and *P. polyxenes* (Appendix 3.1) indicating high intraspecific variability or retained ancestral polymorphism within these species, which is discordant with patterns defined by morphology and geography.

Complete fixation of one clade of *machaon*-like mtDNA was observed in *P. brevicauda*, *P. joanae*, and *P. m. kahli*, while *P. zelicaon* x *machaon* hybrid populations contained that clade as well as *machaon*- and *zelicaon*-like haplotypes more typical of the parental species. All putative hybrids except *P. m. kahli* showed within-group variability, and several haplotypes within the main hybrid clade were shared between species (e.g. hyb11 and hyb12). The main hybrid clade was monophyletic with regard to putative hybrid populations, except for the presence of two *P. machaon* haplotypes: hyb13 from a single *P. m. pikei* Sperling, 1987 specimen collected in Alberta and hyb12 in several *P. m. hudsonianus* specimens collected in Manitoba and Québec (Figure 3.1). Interestingly, the sister lineage to the main hybrid clade is a highly supported, divergent (long branch lengths) clade belonging to two specimens of *P. m. aliaska* Scudder, 1869 collected from northern British Columbia and Alaska (mach2 and mach3) and one specimen of *P. m. hudsonianus* collected from Manitoba (mach8). Additionally, two specimens identified as *P. m. aliaska* (based on morphology, flight period, and habitat) exhibited *zelicaon*-like mtDNA (zel10); upon further examination of these specimens, they showed several intermediate morphological characters between *P. m. aliaska* and *P. zelicaon*.

EF-1α sequences only supported the monophyly of *P. indra*, and gave no resolution for any other species within the group (Figure 3.2a). Due to the ambiguous phylogenetic information content of EF-1α and difficulty in consistently obtaining sequences from older DNA, comprehensive sequencing of all specimens was not pursued.

3.4.3 Microsatellite data

Ten microsatellite loci were genotyped for 130 specimens representing 20 taxa (Table 3.1), and had a total of 225 alleles with a range of 11-32 alleles per locus (Appendix 3.2).

Observed heterozygosity ranged from 0.25 in *P. indra* to 0.67 in *P. zelicaon* x *machaon* and pairwise F_{ST} ranged from 0.010 between *P. zelicaon* and *P. zelicaon* x *machaon* to 0.600 between *P. m. kahli* and *P. indra* (Table 3.3). STRUCTURE predicted an ideal *k* value of *k* = 2, with clusters roughly corresponding to 1) *P. machaon* including *P. m. kahli*, and 2) *P. polyxenes*, *P. brevicauda*, *P. joanae* and *P. zelicaon* (Figure 3.3a); individuals of *P. zelicaon* x *machaon* were split between the two main clusters or, along with some individuals of *P. zelicaon*, were intermediate. Sub-structuring was present for both overall clusters. Within the *polyxenes*/*zelicaon*-like cluster, *k* = 2 separated *P. polyxenes* and *P. brevicauda* from *P. zelicaon* and *P. joanae* (Figure 3.3b), and *k* = 4 identified *P. brevicauda* as having a unique signature (Figure 3.3c). Within the *machaon*-like cluster, *k* = 5 was best supported, which clearly distinguished *P. m. kahli* and *P. m. pikei*, but showed variable ancestry for the other subspecies of *P. machaon* and individuals of *P. zelicaon* x *machaon* from SW Alberta (Figure 3.3d). Two individuals of *P. m. aliaska* were *zelicaon*-like or *zelicaon* x *machaon*-like throughout the STRUCTURE results (Figure 3.3); these were the same two individuals that exhibited *zelicaon*-like mtDNA (Figure 3.2b).

Discriminant analysis of principal components (DAPC) of microsatellite data optimally predicted *k* = 3 genetic clusters, corresponding to one group consisting of *P. machaon* and a few *P. zelicaon* x *machaon*, and two groups sharing all individuals of the other species and a small number of *P. machaon*. Graphically, there is no clear demarcation/separation between these groups; individuals instead fall along a gradient from *P. machaon* to *P. zelicaon* to *P. polyxenes* (Figure 3.4). *Papilio joanae* grouped between *P. polyxenes* and *P. zelicaon*, specimens of the *P. zelicaon* x *machaon* population were between their putative parental species, and *P. brevicauda* clustered closest to *P. polyxenes*, but with some separation from the main gradient. The contributions of individual discriminant functions are displayed as an inset in Figure 3.4, showing that the first function accounts for most of the variation. *Papilio machaon kahli* clusters among *P. machaon* on the first discriminant axis (the x-axis, discriminant function 1), but is separated from the main cluster along the second (the y-axis, discriminant function 2). This separation could be an artifact of small sample size capturing very little within-group variability, but generating additional "individuals" from random alleles observed in *P. m. kahli* maintains this overall pattern.

Considering the overall pattern of genetic clustering and observed intraspecific variation, STRUCTURE and DAPC produce similar results; namely, both analyses show optimal clustering of individuals that separates most *P. machaon* from *P. polyxenes*/*P. zelicaon*, although STRUCTURE illustrates this pattern more explicitly. It is clear that despite their mitochondrial relationships, *P. brevicauda* and *P. joanae* share little nuclear resemblance with *P. machaon*. *Papilio machaon kahli* appears *machaon*-like at a broad level, but along with *P. brevicauda*, is genetically distinct at a finer scale. Finally, *P. zelicaon* x *machaon* shows signatures of high and variable admixture in both STRUCTURE and DAPC, which is exemplified by the occurrence of *machaon*-like, *zelicaon*-like, and hybrid-like individuals present within each of the main mtDNA clades (Figure 3.3, inset).

3.4.4 Morphometrics

Six wing morphometric characters from Sperling (1987) were scored for 127 specimens representing 21 taxa (Table 3.1), and analyzed with multiple correspondence analysis (MCA) (Figure 3.5). Dimension one of the MCA created a gradient between the parental species, stretching from "yellow morph" species (*P. machaon*) to "black morph" species (*P. polyxenes*);

substantial variability was present, however, particularly in *P. machaon*. The putative hybrids showed less within-species variability when compared to the parental species, although smaller sample sizes likely contributed to this pattern. *Papilio brevicauda* and *P. joanae* grouped closely with *P. polyxenes*, which is unsurprising based on their overall appearance (Figure 3.1). *Papilio machaon kahli* was found on the periphery of the *P. polyxenes* cluster, extending towards *P. zelicaon* & *P. machaon*. Finally, *P. zelicaon* x *machaon* hybrids grouped closely within the *P. zelicaon*/*P. machaon* cluster, with one black morph ("nitra") individual extending towards the *P. polyxenes* region. Several individuals of *P. machaon* and *P. polyxenes* also occupy the intermediate space between the main groupings of their respective taxa (four *P. machaon* individuals to the left of x = -0.25, and three *P. polyxenes* individuals to the right of x = -0.4), and correspond to black morph *P. machaon bairdii* Edwards, 1866 and yellow morph *P. polyxenes americus* Kollar, 1849.

3.4.5 Molecular dating

Molecular dating of the complete COI/COII data set resulted in a root age for the divergence of *P. xuthus* from the remaining species of 18.25 (15.29-23.38 95% confidence interval) million years (MY) before present (Figure 3.2, Table 3.4), which, as expected, is quite similar to the age of the respective node found by Condamine *et al*. (2012). This leads to the age of divergence of the main hybrid haplotype clade of 1.60 (0.24-2.45) MY, or in the mid-Pleistocene. The overall topology of this analysis was consistent with that obtained from MrBayes, except the monophyly of the main hybrid group was further broken by the presence of two *P. machaon* haplotypes (Appendix 3.5). Age estimates for main nodes in Figure 3.2 are provided in Table 3.4, and the entire dated tree is shown in Appendix 3.5.

3.5 Discussion

Interspecific hybridization across the *P. machaon* species group in North America is supported by several mutually-reinforcing new lines of evidence. Using mtDNA sequence data, we identified a *machaon*-like lineage shared by four putative hybrid populations or species, confirming earlier findings based on mtDNA restriction-site variation (Sperling and Harrison 1994). In contrast, nuclear markers and morphological characters exhibit variable admixture and intermediacy, ranging from signatures indistinguishable from *P. polyxenes* or *P. zelicaon* to those of a stable hybrid swarm. The one nuclear gene that was sequenced, EF-1α, showed only shared sequence variation among the major species in the group, although it confirmed *P. indra* as the closest outgroup. Hybridization among species would have been facilitated by the repeated glaciations of the Pleistocene, and illustrates the importance that hybridization can have in the evolutionary histories of entire species groups. We first discuss this phylogeographic hypothesis, and then consider the relative roles of hybrid speciation and adaptive introgression in the formation of each of these hybrid lineages.

3.5.1 Pleistocene origins and phylogeography of hybrid lineages

Molecular dating of COI/COII approximates the time of divergence of the main hybrid lineage as mid Pleistocene (Figure 3.2, Table 3.4). The use of a single marker and secondary calibrations make this a rough estimation (e.g. Graur & Martin 2004, but see Forest (2009) regarding the use of multiple secondary calibration points). Nonetheless, with confidence intervals of 1.1-2.8 MY, the initial hybridization events for all hybrid lineages can be confidently placed in the context of the repeated glaciations of the Pleistocene (Mickelson & Colgan 2004). As Sperling & Harrison (1994) hypothesize, at these glacial maxima, the ranges of all three parental taxa would have been forced south, creating new regions of contact or sympatry between *P. machaon* and *P. polyxenes*/*P. zelicaon*, and facilitating hybridization (Figure 3.6). Subsequent glacial contraction likely separated sympatric populations, exposing hybrid remnants to differential introgression from their parental species.

Papilio machaon hudsonianus is the most easterly-distributed subspecies of *P. machaon* in North America (Scott 1986) (Figure 3.1). The fact that *P. m. hudsonianus* mtDNA haplotypes (haplotype mach8) belong to the same mtDNA clade as the putative hybrid taxa supports a previously more widespread distribution for this mtDNA lineage. During or shortly after glacial maxima, *P. m. hudsonianus* from eastern North America would have been likely to come into contact with *P. polyxenes* in or near the current ranges of *P. joanae* and *P. brevicauda* (Figure 3.6). Interestingly, one specimen of *P. m. pikei* also displayed hybrid clade mtDNA (haplotype hyb13), and the sister clade to the main hybrid clade was found in individuals of both *P. m. hudsonianus* and *P. m. aliaska* (clade mach2/3/8). *Papilio machaon pikei* and *P. m. aliaska* have ranges parapatric or sympatric to that of *P. m. hudsonianus* in western Canada (Scott 1986, Bird 1995) (Figure 3.1), and some gene flow between these three subspecies may be expected. However, both *P. m. aliaska* and *P. m. pikei* more commonly have typical *machaon*-like mtDNA haplotypes (e.g. haplotypes mach11, mach12, mach19) rather than those from the hybrid clade, suggesting that the presence of the hybrid clade haplotypes is due to relatively recent mitochondrial gene flow from *P. m. hudsonianus* to *P. m. aliaska* and *P. m. pikei*.

Two individuals that were identified as *P. m. aliaska,* based on general appearance, flight period, and habitat, exhibited *zelicaon*-like signatures for both mtDNA and microsatellites. Morphologically, these individuals clustered closer to *P. zelicaon* x *machaon* individuals, but exhibited a very *P. m. aliaska*-like overall appearance. They were collected alongside many typical *P. m. aliaska* as well as several individuals exhibiting hybrid-like mtDNA at a locality at the southern edge of the range of *P. m. aliaska*. These variable hybrid signatures, all observed on the same day at the same locality, illustrate the scale of evolutionary complexity that is observed in this group. Hybrid-like and more divergent *P. machaon* mtDNA signatures could be the remnants of an ancestral *P. machaon* population lineage once widespread in central and eastern Canada, which took part in hybridization at several locations. More geographically comprehensive sampling of *P. m. hudsonianus* and *P. m. aliaska* from the entirety of their ranges would shed further light on this hypothesis.

Alternative explanations for similar, and in some cases identical, *machaon*-like mtDNA haplotypes found in geographically disjunct putative hybrids are not supported by our data. The likelihood of this repeated geographic pattern arising by neutral chance (i.e. genetic drift: Ballard & Kreitman 1995) is low, and although incomplete lineage sorting can generate phylogenetic patterns that mimic introgression, it would not be expected to leave any appreciable phylogeographic signal (Funk & Omland 2003, McKay & Zink 2009). *Wolbachia*, a bacterial endosymbiont that can cause cytonuclear discordance (e.g. Jiggins 2003), has not been detected in the species group (Dupuis *personal observation*). Finally, if neutral processes such as drift or founders' effects were to fix introgressed haplotypes in putative hybrid populations, allelic (or haplotype) diversity would be expected to be low (Wilson & Bernatchez 1998), which was not observed.

3.5.2 Hybrid speciation in the P. machaon *group*

Our data supports hybrid origins for four more-or-less distinct populations of the *P. machaon* species group. These lineages arose from similar situations, namely mating between *P.*

machaon (likely *P. m. hudsonianus*) and *P. polyxenes*/*P. zelicaon*, but interestingly this has produced different outcomes in their current genetic composition and ecological associations. This repetition provides a unique framework to compare the outcomes of hybridization with regard to hybrid speciation and adaptive introgression. Both processes require hybridization between distinct biological entities, and generally result in some kind of cytonuclear discordance. While the distinction between hybrid speciation and adaptive introgression may seem arbitrary outside of speciation theory, biodiversity estimation and conservation generally rely on species as a common unit of diversity. In this setting, it is important whether adaptive traits have introgressed from one species to another or two species have hybridized to create a third (e.g. DeMarais *et al*. 1992, Haig *et al.* 2004, Monzón *et al.* 2014).

The key to distinguishing hybrid species from cases of adaptive introgression is to diagnose unique hybrid traits or combinations that facilitate reproductive isolation of hybrids from *both* parents (Abbott *et al.* 2013). Although genomic admixture and morphological intermediacy are conceptually intuitive indicators of potential hybridization, they can be difficult to tease apart from ancestral polymorphism and recent gene flow (Hedrick 2013), and by themselves may not be enough to define a species as a homoploid hybrid (Abbott *et al.* 2013). This is especially true in cases of backcrossing to a single parent species (Mallet 2007), as would be expected with the *P. machaon* complex. Therefore, ecological evidence of reproductive isolation from both parents is often used in defining hybrid species (e.g. Gompert *et al.* 2006, Schwarz *et al.* 2007), although this isolation need not be absolute (e.g. Hermansen *et al.* 2011, Kunte *et al.* 2011, Stemshorn *et al.* 2011).

Here we focus on two criteria for defining a lineage as a hybrid species: 1) a hybrid species must have shared characteristics with both parents, but also some level of distinctiveness that facilitates diagnosis of the hybrids; and more importantly, 2) a hybrid species must have some novel characteristics that facilitate reproductive isolation from the parental species (Abbott *et al.* 2013). For the purposes of this study, meeting both of these criteria provides support for the lineage in question being a hybrid species; while meeting only one criteria (e.g. displaying genealogical discordance or introgression, but no characteristics of reproductive isolation) is insufficient evidence, and is more likely a scenario involving adaptive introgression in the history of the lineage. Although this framework greatly simplifies a conceptually complicated and difficult task (Abbott *et al.* 2013), it is appropriate given the nature of this genetic data (non-genomic) and the paucity of ecological information for these butterflies, which are often difficult to find. To assist in this evaluation, we have compiled pertinent ecological traits known to facilitate reproductive isolation in Appendix 3.4, and have summarized this information, as well as our morphological and genetic conclusions, in Figure 3.7.

3.5.2.1 Papilio brevicauda

To lepidopterists familiar with swallowtails, *P. brevicauda* is a clearly diagnosable entity: its combination of *polyxenes*-like (black morph, orange undersides of hind wings) and *machaon*-like traits (short hindwing tails and shorter, rounded forewings) is distinctive and supports its widely-recognized species status. Only the *polyxenes*-like characters were included in our morphological analysis, due to the limited quality of voucher specimen wings and subsequent missing data, which can account for its indeterminate placement in the MCA. Genetically, *P. brevicauda* also displays characteristics of both *P. machaon* and *P. polyxenes*, as well as some degree of distinctiveness in nuclear markers at a fine-scale, supporting its consideration as a hybrid species. However, it appears to have no clear ecological separation from either parental species that would provide reproductive isolation from them (Figure 3.7) (Scott 1986, Layberry *et al.* 1998, Ferguson 1955, Scott 1986, Scriber 2007), although one unknown in this ecological assessment is the eastern range limit of *P. m. hudsonianus*. Historic and potentially ongoing introgression of *P. m. hudsonianus* genes into a northern-adapted, coastal lineage of *P. polyxenes* could account for *P. brevicauda*'s morphological and genetic intermediacy, but it requires that that *machaon*-like mtDNA experienced a selective sweep through the population (Bazin *et al.* 2006). However, with the data at hand, the evidence for novelty of putative hybrid characteristics or reproductive isolation is not substantial, and so *P. brevicauda* does not fully qualify as a hybrid species. Comprehensive geographic sampling, particularly at the western and southern edges of the range of *P. brevicauda* (where it may be sympatric or parapatric with *P. m. hudsonianus* and *P. polyxenes*, respectively), should clarify its status.

3.5.2.2 Papilio joanae

Unlike *P. brevicauda, P. joanae* is often morphologically indistinguishable from *P. polyxenes asterius* Stoll, 1782 (Scott 1986, Schweitzer *et al.* 2011), and has only a handful of *machaon*-like traits. Except for mtDNA, its genetic characteristics are more *polyxenes*-like, although at fine scales it is somewhat *zelicaon*-like (Figure 3.3 and 3.4). Many of *P. joanae*'s ecological traits are shared with *P. p. asterius* (Heitzman 1973, Scott 1986), except for its affinity for closed forests and cedar glades (Heitzman 1973), rather than the open habitats (fields, exposed hilltops) that are used by the rest of the species group. This strict use of forest habitats nonetheless provides substantial separation of the two species, as *P. joanae* larvae are only found on hosts within forest habitats and *P. p. asterius* larvae are found only in open areas (Heitzman *personal communication*). Interestingly, *P. m. hudsonianus* is the only other North American member of the species group that frequents forest edges and shaded habitats, and even oviposits in shaded areas (Dupuis *personal observation*), although it uses different hosts than *P. joanae*.

The novelty of this *P. m. hudsonianus*-like ecological characteristic, which contributes to reproductive isolation of *P. joanae* from *P. polyxenes*, supports the hypothesis that *P. joanae* is indeed a hybrid species. This isolation is similar to ecological separation in several other North American hybrid butterfly species (Gompert *et al.* 2006, Kunte *et al.* 2011). Adaptive introgression of *machaon*-like genes into *P. polyxenes* is also possible, but this alternative explanation is less likely. The nearest populations with similar mtDNA haplotypes are now over 1000 kilometers to the north. At finer scales, *P. joanae* displays *zelicaon*-like as well as *polyxenes*-like nuclear characteristics (Figures 3.3 and 3.4), suggesting older hybridization between *P. m. hudsonianus* and the common ancestor of *P. polyxenes* and *P. zelicaon*. For these reasons, we conclude that *P. joanae* is a homoploid hybrid species that is reproductively isolated from its parents via behavioral separation. This may be an important consideration for future conservation prioritization of *P. joanae* (Schweitzer *et al.* 2011).

3.5.2.3 Papilio machaon kahli

Papilio machaon kahli is the most enigmatic of the putative hybrids considered here. Morphologically, it is intermediate between *P. machaon* and *P. polyxenes* (Figure 3.5), and lepidopterists have found it difficult to distinguish it from *P. polyxenes* based on overall appearance (Klassen *et al.* 1989, Layberry *et al.* 1998). Genetically, both mtDNA and nuclear DNA show ties to *P. machaon* (Figures 3.2 and 3.3a), but at a finer scale *P. m. kahli* is quite distinct from the rest of the species group (Figures 3.3d and 3.4). However, more samples are needed to elaborate this potential unique signature. We find little ecological support for any traits that would provide reproductive isolation from *P. machaon* or *P. polyxenes*; the use of its main host, *Zizia aptera* A. Gray, may provide separation from *P. m. hudsonianus*, although the range of hosts used by the latter subspecies is unclear (Sperling 1987, Tyler *et al.* 1994). Interestingly, in the past 100 years there may have been a decline in the presence of *kahli*-like individuals throughout the small range of this taxon, and an increase in the presence of *P. p. asterius* (Sperling 1987). Unfortunately our study includes only one specimen collected more recently than 1990 (Appendix 3.1), so we cannot attest to the current status of that trend, but microsatellite data clustering shows little sign of *polyxenes*-like ancestry. Overall, we find no support for hybrid species status, and, based on the data at hand, we consider it most likely that *P. m. kahli* is a transitional population of *P. m. hudsonianus* experiencing adaptive introgression from *P. polyxenes*. The geographically limited range of this lineage was likely instrumental in its taxonomic recognition as a subspecies.

3.5.2.4 Papilio zelicaon *x* machaon

Populations of *P. zelicaon* x *machaon* in SW Alberta display a very different scenario compared to other potential hybrid taxa, in that hybrids are parapatric with both parental taxa, and all three mtDNA types are found in the same populations. Nuclear admixture is also variable among individuals, resembling early-generation hybrids (F1, F2, backcross) and both *P. machaon* and *P. zelicaon*. Morphological variation in these populations mirrors the nuclear admixture, although black ("nitra") morphs resembling *P. polyxenes* are also observed (Figure 3.7). Host plants and habitat preferences may provide hybrids with some ecological separation from *P. m. dodi* McDunnough, 1939, which feeds strictly on *Artemesia dracunculus* L. in arid river valleys, but not from *P. zelicaon* (Sperling 1987); adult hilltopping locations of *P. m. dodi* and *P. zelicaon* are often close enough to each other that both species can be found in the other's respective habitat. The southern range limit of *P. m. hudsonianus* is unclear, although *hudsonianus*-like specimens have been observed in southern Alberta in the vicinity of hybrid populations (Bird *et al.* 1995). Whether these rare occurrences represent migrants from farther north or persisting populations is unknown. If they do represent the latter, then host choice may also foster ecological isolation between *P. m. hudsonianus* and *P. zelicaon* x *machaon* individuals.

The presence of mitochondrial, nuclear, and morphological intermediates at varying stages of evolutionary separation indicates that these populations represent a stable hybrid swarm (Nolte & Tautz 2010, Latch *et al.* 2011), and are far from being a distinct hybrid species. Predominant *zelicaon*-like ancestry is indicated with nuclear markers (Figures 3.3 and 3.4), but more comprehensive geographic sampling would clarify the situation. Interestingly, morphologically similar intermediates have been collected from the area since the early twentieth century before significant agricultural habitat changes (Sperling 1987), indicating that there was no anthropogenic influence on the initial formation of a hybrid population. Whether this situation will progress to hybrid speciation, as seen with *P. joanae*, will depend on the development of mechanisms for ecological and reproductive isolation from both parental species.

3.5.3 Other lepidopteran hybrids

New World Lepidoptera have had disproportionate influence on the study of hybridization, due in part to their high diversity and general appeal, and these systems provide a rich foundation to compare to that of the *P. machaon* group. *Lycaeides* butterflies in western North America share many similarities with the *P. machaon* group, particularly in the multifarious nature of hybridization observed in the group (Gompert *et al.* 2014). Widespread historic admixture throughout *Lycaeides* is reminiscent of the *P. m. hudsonianus*-like signatures observed across North America in putative hybrid lineages. Additionally, ecological separation from parental taxa has allowed some hybrids to persist in novel habitats, akin to *P. joanae* (Gompert *et al.* 2006). *Heliconius* butterflies in Central and South America regularly hybridize (e.g. Mavárez *et al.* 2006, Mallet *et al.* 2007, Nadeau *et al.* 2012), similarly to members of the *P. machaon* complex, and abundant ecological and genomic data has revealed promiscuous exchange of genes controlling protective color-pattern between hybridizing species (Dasamahaptra *et al.* 2012). However, contention regarding the hybrid origins of some *Heliconius* species (Brower 2011) emphasizes the importance of addressing the fine differences between hybrid speciation and adaptive introgression. Within the Papilionidae, ecological and genomic data support the hybrid status of *Papilio appalachiensis* Pavulaan & Wright, 2002 (Scriber & Ording 2005, Zhang *et al*. 2013, Cong *et al.* 2015), where it is also clear that ecological separation has aided reproductive isolation from the parental species (Kunte *et al.* 2011), again similarly to *P. joanae*. With growing insight into the potential for hybridization to encourage diversification and adaptation (e.g. Gompert *et al.* 2014, Dasmahapatra *et al.* 2012), we are confident that continued work on the *P. machaon* group will facilitate this understanding, and support Clarke and Sheppard's (1955) assertion "that the Machaon-group provides some of the most suitable material ever investigated in animals for studying the process of speciation in detail."

3.6 Conclusions

Our results demonstrate a case of repeated reticulate evolution within a species complex of swallowtail butterflies in North America. We have documented three geographically separated cases of cytonuclear discordance where mtDNA is completely fixed in hybrid lineages (*P. brevicauda*, *P. joanae*, and *P. m. kahli*), and one case where both hybrid and parental mitochondrial haplotypes occur (*P. zelicaon* x *machaon*). Excluding the parental-like mtDNA haplotypes found in *P. zelicaon* x *machaon* populations, all of the mtDNA of hybrids is likely derived from a single *machaon*-like lineage resembling *P. m. hudsonianus*, despite geographic separation of these populations from *P. machaon* and from each other. Nuclear markers show variable signatures ranging from almost completely paternal characteristics, to high levels of admixture and potentially unique hybrid signatures; morphological characters also show variable levels of intermediacy. The divergences of these hybrid lineages were dated to the mid-Pleistocene, indicating an important role for glacial refugia in their formation. Despite similar hybrid origins (*P. machaon* hybridizing with *P. polyxenes*/*P. zelicaon*), these lineages have followed distinct evolutionary trajectories leading to diverse outcomes, from hybrid speciation (*P. joanae*) and potential adaptive introgression (*P. brevicauda* and *P. m. kahli*) to stable hybrid swarms (*P. zelicaon* x *machaon*). These results add to a growing recognition of the evolutionary importance and complexity of hybridization in generating biodiversity.

Table 3.1 Species and specimens examined in this study.

Species	Subspecies	Seq	Msat	Morph
P. machaon	*aliaska* Scudder, 1869	13(1)	12	12
	bairdii Edwards, 1866	7(1)	4	7
	dodi McDunnough, 1939	7	7	7
	gorganus Fruhstorfer, 1922	2(1)	2	2
	hippocrates Felder & Felder, 1864	2(1)	2	3
	hudsonianus Clark, 1932	7	6	6
	oregonius Edwards, 1876	1(1)	2	3
	pikei Sperling, 1987	8(1)	6	6
	kahli Chemrock & Chemrock, 1937	3	4	3
P. polyxenes	*americus* Kollar, 1849	2(1)	3	4
	asterius Stoll, 1782	8(1)	11	9
	coloro Wright, 1905	5(1)	6	6
	stabilis Rothschild & Jordan, 1906	1(1)	1	1
P. zelicaon Lucas, 1852		25(4)	24	19
P. zelicaon x *machaon*		23(1)	23	20
P. brevicauda	*brevicauda* Saunders, 1868	4	4	4
	gaspeensis McDunnough, 1934	3(2)	2	3
P. joanae Heitzman, 1973		6(2)	7	7
P. hospiton Gené, 1839		1(1)	0	1
P. indra	*indra* Reakirt, 1866	3(3)	3	3
	kaibabensis Bauer, 1955	1(1)	1	1
P. xuthus Linnaeus, 1767		1(1)	0	0
Total		*133(25)*	*130*	*127*

Sample sizes for COI/COII sequence data (SEQ), microsatellites (MSAT), and morphological characters (MORPH). Number in parentheses in sequence data column corresponds to EF-1α.

Table 3.2 Summary results from maximum parsimony and maximum likelihood analyses.

Gene	Data set	Characters		MP				ML Score
		Inf.*	Total	# Trees	Score	CI	RI	
COI/COII	Extended	164	2288	15186400	463	0.793/0.668	0.895	-5369.1201
	Complete	24	306	28	71	0.817/0.675	0.882	-753.4862
EF-1α	Extended	16	1010	22976344	86	0.919/0.720	0.877	-1927.2094
	Complete	9	418	859	42	0.952/0.833	0.957	-831.0601

Extended and complete data sets are included. Abbreviations: CI: consistency index, RI: retention index, Inf.*: parsimony informative characters, MP: maximum parsimony, ML: maximum likelihood. For CI values, the first reported number includes uninformative characters, and the second excludes them.

Table 3.3 F_{ST} and heterozygosity values based on microsatellite data.

	polyx.	*brevi.*	*joanae*	*zelicaon*	*zel x mac*	*kahli*	*mach.*	*indra*
P. polyxenes	0.47/0.81	-	-	-	-	-	-	-
P. brevicauda	0.114	0.51/0.57	-	-	-	-	-	-
P. joanae	0.068	0.223	0.61/0.78	-	-	-	-	-
P. zelicaon	0.047	0.143	0.049	0.62/0.87	-	-	-	-
P. zelicaon x machaon	0.058	0.181	0.065	0.010	0.67/0.84	-	-	-
P. m. kahli	0.245	0.456	0.309	0.202	0.217	0.44/0.41	-	-
P. machaon	0.113	0.216	0.096	0.067	0.047	0.236	0.50/0.82	-
P. indra	0.281	0.481	0.375	0.239	0.291	0.600	0.288	0.25/0.43

F_{ST} comparisons based on microsatellite data between species in bottom triangle, heterozygosity values within each species on diagonal (observed/expected).

Table 3.4 Age estimates and 95% confidence intervals of major nodes shown in Figure 3.2.

Clade #	Age estimate	95% CI
1	18.25	15.29-23.38
2	7.07	5.02-9.01
3	5.12	3.77-7.01
4	1.60	0.24-2.45
5	3.46	0.51-6.71
6	1.51	0.08-3.73
7	1.46	0.07-3.67

All ages in millions of years.

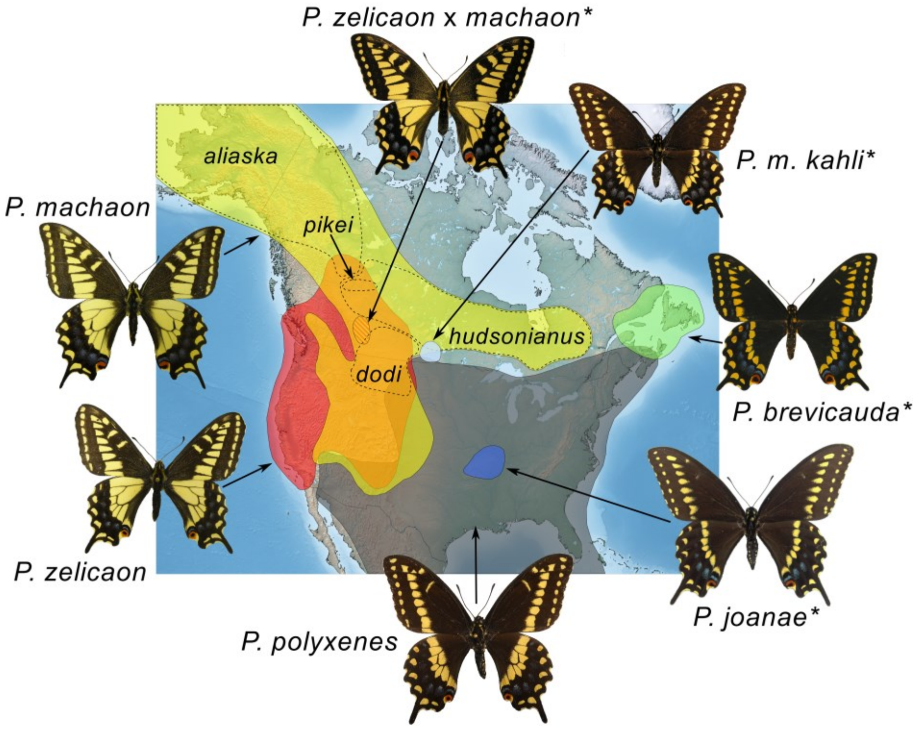

Figure 3.1 Generalized range map of current distributions of the *Papilio machaon* species complex in North America. Putative hybrid taxa are indicated with an asterisk. Dashed lines indicate approximate ranges of *P. machaon* subspecies pertinent to the current study. *Papilio joanae* holotype photograph: J. Tewell.

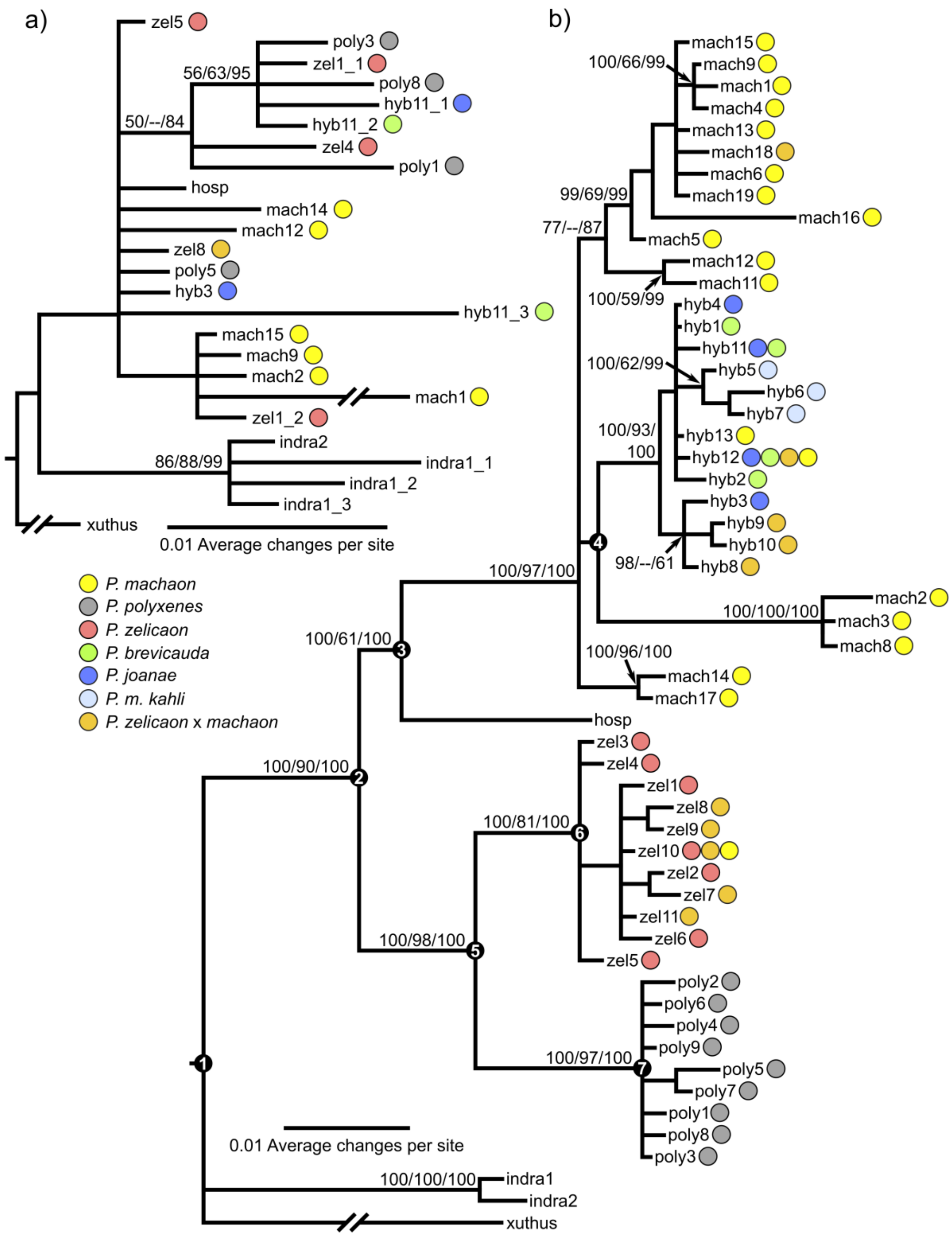

Figure 3.2 Fifty percent majority rule consensus trees constructed using Bayesian Inference. a) Extended EF-1α sequence data for 25 genotypes, and b) extended COI/COII sequence data for 54 haplotypes. Numbers above branches represent bootstrap support (if >50%) for maximum

parsimony (first number), maximum likelihood (second number) and Bayesian posterior probability values (third number), for nodes that are consistently resolved among search criteria. Colors in circles correspond to the species possessing each haplotype. EF-1α genotype names reflect the corresponding COI/COII haplotype displayed by each specimen, with an added underscore and number indicating cases where specimens that shared COI/COII haplotypes had more than one different EF-1α genotype. Age estimates using COI/COII data for major nodes (numbered black circles) are provided in Table 3.4.

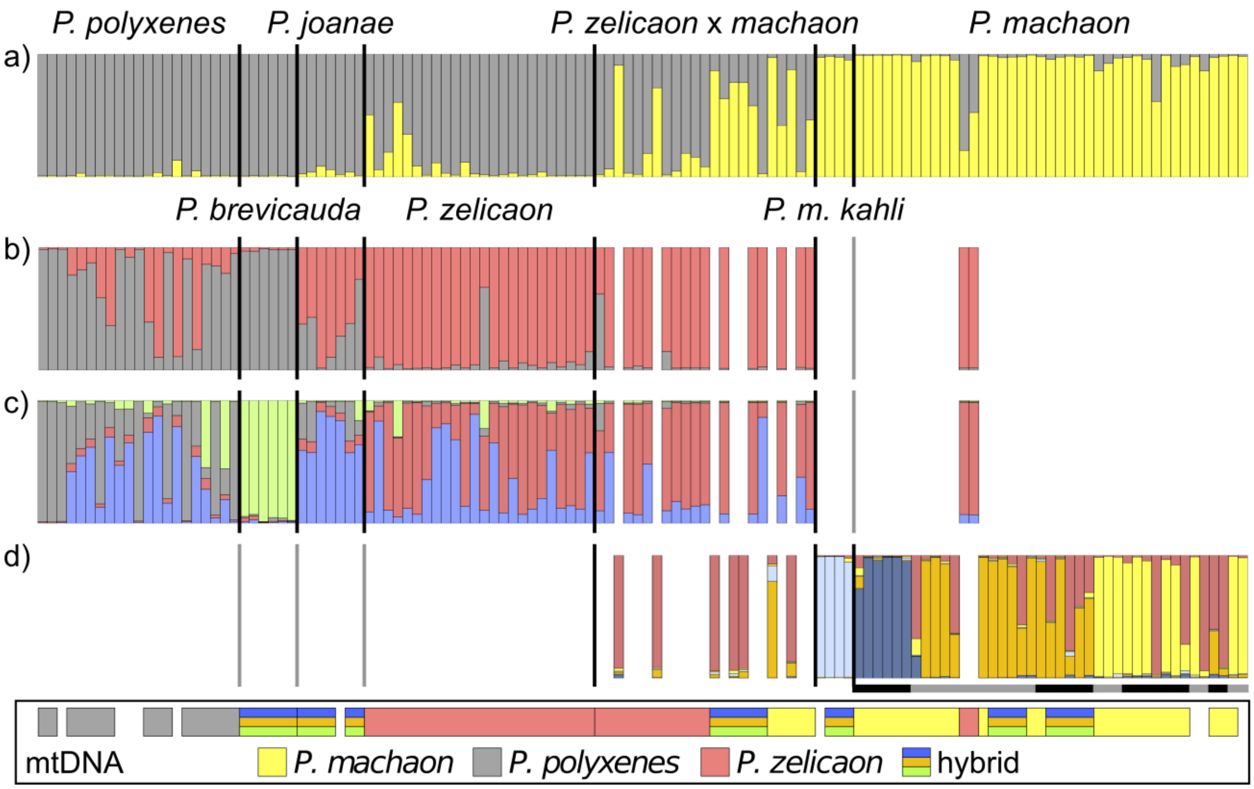

Figure 3.3 STRUCTURE results for microsatellite genotype data. a) $k = 2$ based on overall dataset; b) and c) $k = 2$ and $k = 4$, respectively, for the *polyxenes*/*zelicaon*-like cluster from the overall $k = 2$; and d) $k = 5$ for the *machaon*-like cluster from the overall $k = 2$ (individuals with ≥70% *P. machaon* ancestry). Inset indicates the major mtDNA clade (*P. machaon*, *P. polyxenes*, *P. zelicaon*, or the main hybrid clade within the *P. machaon* clade) for each individual (gaps indicate specimens genotyped for microsatellites that were not sequenced for COI/COII). Alternating black and grey bars above and below the *P. machaon* portion of d) indicate subspecies; from left to right: *P. m. pikei*, *aliaska*, *hudsonianus*, *bairdii*, *dodi*, *oregonius*, *gorganus*, and *hippocrates*.

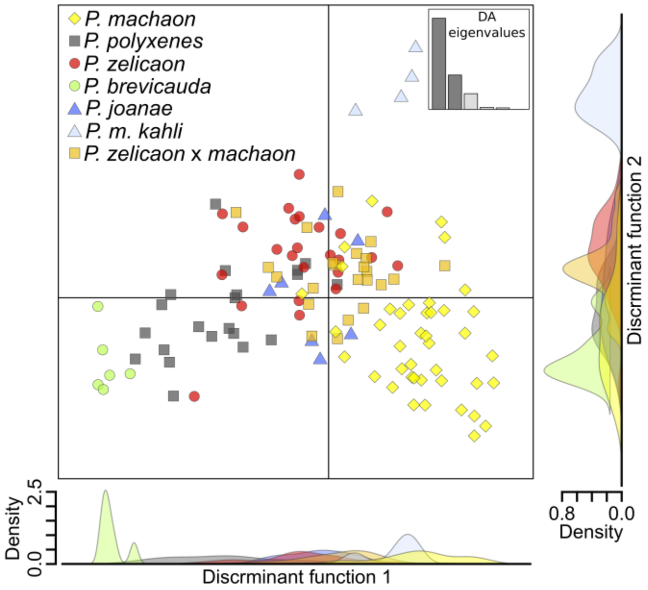

Figure 3.4 DAPC of putative hybrids and parental taxa generated from microsatellite data. Inset illustrates the relative contribution of individual discriminant functions (DFs) to overall variability, and the density plots of the two plotted DFs are shown outside of the main plot.

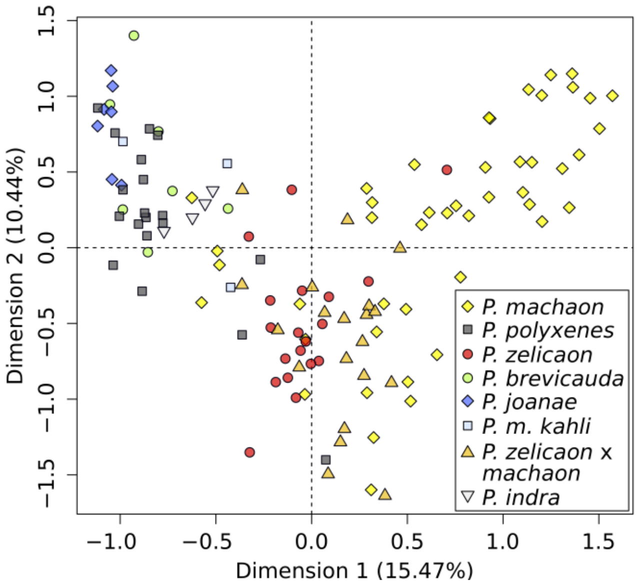

Figure 3.5 Multiple correspondence analysis of six morphological characters. Plotted using the *jitter* function to improve visualization of overlapping points (non-*jittered* results shown in Appendix 3.9).

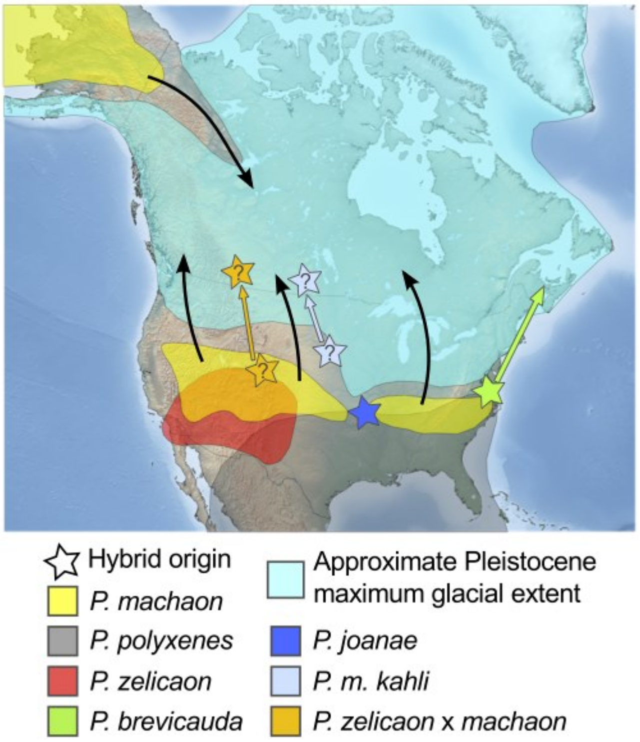

Figure 3.6 Approximate maximum glacial extent during the early- to mid-Pleistocene, hypothesized glacial refugia and hybrid origins. Arrows indicate hypothesized postglacial recolonization routes of hybrids (colored arrows), and parental species (black arrows). Hybrid origins with question marks indicate origins with less geographic certainty.

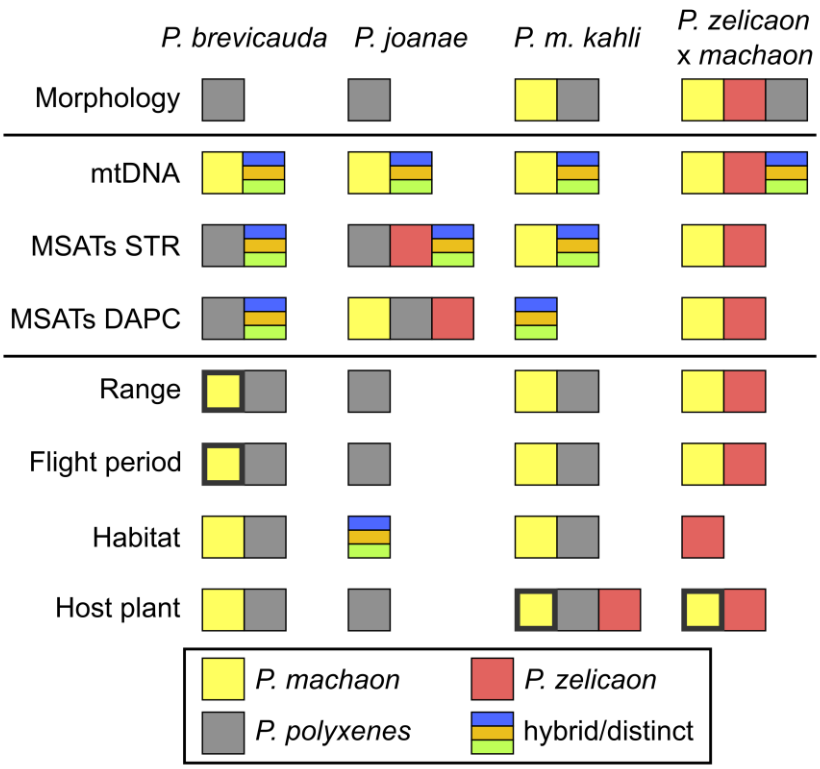

Figure 3.7 Summary of parental similarity and distinctiveness of four putative hybrid lineages with morphological, genetic, and ecological data. Colored boxes denote similarity or clustering with parental species, or distinctness of the hybrid lineage. Morphology is based on clustering in morphological analysis (MCA). mtDNA is based on mitochondrial DNA clades (note that the unique hybrid clade is identified as both distinct and as *P. machaon*-like). MSATs STR is based on summarized ancestry at the finest subpopulation scale in STRUCTURE analyses, and MSATs DAPC on summarized clustering in DAPC analyses. Range shows sympatry or parapatry with parental species, flight period shows overlapping adult flight period, habitat is based on a general assessment of shared habitat type (forest openings, strict hilltopping behavior, under forest cover, etc.), and host plant shows shared larval host. Boxes with wide outlines indicate uncertainty in particular characteristics. Ecological information is presented in more detail in Appendix 3.4.

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Chapter 4

Hybrid dynamics and identification in a species group of swallowtail butterflies

4.1 Summary

Hybrid zones provide unique natural laboratories for studying mechanisms of evolution. But identification and classification of hybrid individuals (F1, F2, backcross, etc.) can be complicated by population changes over time as well as variation in the information provided by different markers. Here, we use multiple genetic markers (mitochondrial DNA, microsatellites, and genome-wide single nucleotide polymorphisms) to: a) reexamine population structure in a hybrid zone between two species of swallowtail butterflies in western Canada, *Papilio machaon* and *P. zelicaon*, to test whether their hybrid dynamics remain the same as found 30 years ago using morphology and allozymes; and b) compare alternative hybrid identification and classification methods for genetic datasets. Overall, we found high differentiation between the two parental species, corroborating previous research. However, we observed fewer hybrid individuals in the main zone of hybridization, depending on the genetic marker considered. Comparison of methods with simulated datasets generated from our data showed that single nucleotide polymorphisms were more powerful than microsatellites for both hybrid identification and classification. However, there was also substantial variability among comparisons, underlining the value of multiple markers and methods for identifying and classifying hybrid individuals in evolutionarily dynamic systems.

4.2 Introduction

Hybrid zones between genetically distinct but closely related species provide unique opportunities to investigate the mechanisms that control gene flow and the dynamics of reproductive isolation (Hewitt 1988, Abbott *et al.* 2013). Genomic research on hybrids has supported the concept of species boundaries as porous units (Sperling 2003, Dasamahapatra *et al.* 2012, Gompert *et al.* 2012, Harrison & Larson 2014), and shown that hybridization can facilitate adaptation and speciation at both small and large genomic scales (Dasamahapatra *et al.* 2012, Rieseberg *et al.* 2003). Despite growing understanding of hybrid zone dynamics, however, the complexity of such zones of genomic recombination precludes straightforward identification and classification of hybrid individuals in nature (e.g. Lowe *et al.* 2015 *and references therein*, Mandeville *et al.* 2015).

Traditional classification of hybrid individuals uses terminology from early plant and animal breeding experiments: parental, filial (F1, F2, etc.), and backcross (e.g. Bateson & Saunders 1902). This ancestry-based classification can inform a quantitative hybrid index of the proportion of parental characteristics in hybrids, with parent taxa assigned a value of zero or one, and F1 hybrids a value of 0.5. Such a hybrid index is appealingly simple and recognizes that hybrid individuals in wild populations may form a continuum, but ignores genotypic characteristics that could differentiate later generation hybrids and backcrosses (Jiggins & Mallet 2000, Fitzpatrick 2012). Consequently, other approaches to hybrid characterization have gained popularity, including model-based methods that incorporate known parental allele frequencies (Buerkle 2005, Gompert & Buerkle 2010), heterozygosity (Fitzpatrick 2012), and Bayesian estimates of multilocus ancestry (Pritchard 2000, Anderson & Thompson 2002). Increased accuracy in characterization and classification of hybrid individuals is pivotal to understanding and predicting outcomes of reproductive isolation in hybrid systems (e.g. Mandeville *et al.* 2015) as well as facilitating conservation management (DeMarias *et al.* 1992, Haig *et al.* 2004, Monzón *et al.* 2014, Hamilton & Miller 2015).

The *Papilio machaon* species complex of swallowtail butterflies provides a rich foundation for studying hybridization. Fertile hybrids can be generated from lab crosses between virtually all species in the group (e.g. Clarke & Sheppard 1954, Ae 1979), and several distinct species, subspecies, and populations in North America may be of hybrid origin (Sperling 1987, Sperling 1990, Sperling & Harrison 1994, Dupuis & Sperling 2015). Here we focus on the two species in the group that are found in Alberta, *Papilio machaon* and *P. zelicaon*. *Papilio machaon* has a Holarctic distribution, with three subspecies generally recognized in Alberta: *P. m. dodi*, *P. m. pikei*, and *P. m. hudsonianus* (Scott 1986, Sperling 1987, Layberry *et al.* 1998, Pelham 2008; but see Guppy and Shepard 2001). The first two subspecies are specialists of arid river valleys, where larvae feed on tarragon, *Artemesia dracunculus,* on steep, eroding riverbanks, and adults hilltop (mate-locating behavior by congregating on topographical prominences, Shields 1967) along high edges of river valleys (Scott 1986). *Papilio machaon dodi* is found in southern Alberta, while *P. m. pikei* is restricted to the Peace River valley of north central Alberta and adjacent British Columbia (Bird *et al.* 1995). The third subspecies, *P. m. hudsonianus* occurs infrequently in Alberta in open clearings and hilltops in boreal forest (Scott 1986), where its larvae feed on coltsfoot, *Petasites palmatus* Aiton (Sperling 1987, Layberry *et al.* 1998, Dupuis *personal observation*). *Papilio zelicaon* has a Nearctic distribution extending north across most of Alberta, where only one subspecies is recognized (Scott 1986, Sperling 1987). Habitat associations of *P. zelicaon* in Alberta are variable, with adults displaying strong hilltopping behavior on prairie hilltops and Rocky Mountain foothills, and larvae feeding on the parsley family (Apiaceae), mainly *Zizia aptera, Heracleum lanatum* Bartr. and various species of *Angelica* L. and *Lomatium* Raf. (Sperling 1987, Bird *et al.* 1995, Layberry *et al.* 1998).

Papilio machaon and *P. zelicaon* are morphologically similar, but can be differentiated by wing shape and varied yellow and black markings, particularly an eye-spot near the hindwing tails (Figure 4.1). Morphologically intermediate specimens, however, are common in the foothills of southwestern Alberta, where they can be more frequent than putatively pure *P. machaon* and *P. zelicaon* and have been collected for over 100 years (Sperling 1987, Sperling 1990). Based on investigation of morphological characters and allozymes, Sperling (1987) concluded that intermediate individuals in some areas constituted a hybrid swarm. Regional differences in the frequency of hybrid individuals varied from >90% in southwestern Alberta to 20-40% in central Alberta and <5% elsewhere in Alberta and British Columbia. Despite geographic proximity between populations of *P. zelicaon* and *P. m. dodi* or *P. m. pikei* in the Red Deer River and Peace River valleys, the *machaon*-like signatures found in hybrid populations more closely matched those of *P. m. hudsonianus* in northern Manitoba (Sperling 1987). Dupuis & Sperling (2015) found similar signatures of hybridization using mitochondrial DNA (mtDNA) and microsatellite marker variation across the species complex in North America. In this survey, hybrids in southwestern Alberta displayed mtDNA typical of *P. machaon*- and *P. zelicaon*, as well as a mtDNA lineage that was widespread in putative hybrid lineages across North America. Microsatellites also showed high variability in these hybrid populations, with signatures of both parental taxa (Dupuis & Sperling 2015).

Here we provide a fine scale test of population structure and hybridization between *P. machaon* and *P. zelicaon* across Alberta and adjacent northeastern British Columbia. We focus on four geographic areas with varying degrees of hybridization, based on Sperling (1987), Sperling (1990) and Sperling & Harrison (1994). First, we test whether new genetic data (microsatellites and mtDNA) show the same population structure found 30 years ago by Sperling (1987). Second, we genotype single nucleotide polymorphisms (SNPs) in a subset of individuals to test whether the same genomic mosaicism of hybrids is shown in genome-wide SNPs as in microsatellites. Finally, to better evaluate differences between our results and those of Sperling (1987), we compare several methods for identifying and classifying hybrid individuals, using microsatellite and SNP data.

4.3 Methods

4.3.1 Specimen collection

Specimen collections were divided into four regions: 1. Red Deer River, 2. Peace River, 3. Swan Hills, and 4. Foothills of the Rocky Mountains (Figure 4.2, Appendix 4.1). The two river regions are characterized by eroding prairie habitat with *P. machaon* along high riverbanks, and partially forested hilltops with *P. zelicaon* away from the river valleys. The Foothills and Swan Hills are characterized by rolling topography and exposed locations where adults hilltop. Both adults and larvae were collected, depending on access to habitat for each life stage. Butterflies were collected at hilltop locations with an aerial net, then live frozen at -70°C. Caterpillars were collected on host plants adjacent to hilltop locations, and reared to the adult stage on clipped host plant material before being frozen. Provincial parks in Alberta and British Columbia were collected under permit numbers 10-097 and 105180, respectively, and owner's permission was obtained for collecting on any private land.

4.3.2 Mitochondrial DNA sequence data

An 871 base pair (bp) region of *cytochrome oxidase subunit I* (COI) was targeted with the primers Jerry and Pat (Caterino and Sperling 1999), and PCR and sequencing protocols followed Dupuis & Sperling (2015). This sequence gave lineages corresponding to *P. machaon*, *P. zelicaon*, and a third hybrid clade identified by Dupuis & Sperling (2015). Sequences were consolidated and aligned using Chromaseq v1.01 (Maddison & Maddison 2014) implemented in Mesquite v2.75 (Maddison & Maddison 2011) and ClustalW v2.0.12 using default settings (Larkin *et al.* 2007). All sequences were inspected manually for errors, with unambiguous alignment to a sequence of *P. xuthus* [GenBank AF043999.1] that served as an outgroup for phylogenetic analyses.

Sequence data was collected from 822 individuals (Appendix 4.1), and MacClade v4.08a (Maddison & Maddison 2005) was used to consolidate strictly redundant haplotypes. We trimmed the ends of the initial alignment to eliminate variable missing data resulting from poor sequencing, creating an alignment of 594 bp (22 unique haplotypes). Although this decreased the number of phylogenetically informative characters, overall topology and support measures were consistent with longer alignments (results not shown). Gene trees were constructed with unweighted, unordered maximum parsimony (MP: Fitch 1971) and Bayesian inference (BI), in PAUP* v4.0b10-x8 (Swofford 2002) and MrBayes v3.2 (Ronquist *et al.* 2011), respectively. One hundred bootstrap replicates (Felsenstein 1985) were performed for both heuristics with the following parameters: PAUP*: random sequence addition (retaining 100 trees per replicate, of minimum score 1) and tree-bisection and reconnection branch swapping (Swofford & Olsen 1990); MrBayes: 2,000,000 generations (sampling every 100) with default chain settings/temperatures, no additional priors, and removing 25% of sampled trees for burn-in (which was visually confirmed as appropriate in the log-likelihood overlay plot). The Akaike Information Criterion was used (Akaike 1974) in jModelTest v2.1.1 (Darriba *et al.* 2012, Guindon & Gascuel 2003) to select the general time reversal model of substitution (GTR: Tavaré 1986) + I, which was implemented during the BI. Additionally, potential scale reduction factors and average standard deviation of split frequencies were observed during the BI to ensure that independent simulations were converging.

4.3.3 Microsatellite data

Ten microsatellite loci (Zakharov & Hellman 2007) were amplified and genotyped as in Dupuis & Sperling (2015) for 821 individuals. Descriptive statistics (allele frequencies, heterozygosity, inbreeding coefficient, pair-wise F_{ST}) were calculated in GenoDive (Meirmans & Van Tienderen 2004) for populations containing at least seven individuals (38 populations, 781 individuals). GenoDive was also used to calculate pair wise population differentiation between these 38 populations using the analysis of molecular variance (AMOVA) F_{ST} method (Excoffier *et al.* 1992, Michalakis & Excoffier 1996) and 999 permutations, tested for Hardy-Weinberg equilibrium (HWE) using least-squares AMOVA and 100,000 permutations. Bonferroni correction was used for all tests involving multiple comparisons. Gene diversity among individuals in a population (1 - Q-inter) was calculated with GENEPOP v1.2 (Raymond & Rousset 1995, Rousset 2008). We tested for signatures of recent population bottlenecks and expansions with BOTTLENECK v1.2.02 (Corneut & Luikart 1996, Piry *et al.* 1999) using both a strict stepwise mutation model (Kimura & Ohta 1978) and a two-phase model (which may be more appropriate for microsatellites: Di Rienzo *et al.* 1994), with 95% single-step mutations, 5% multiple-step mutations (with a variance of 12, as recommended by Piry *et al.* 1999), and one thousand iterations. We tested for deviations of observed heterozygosity from expected values at the drift-mutation equilibrium with the Wilcoxon sign rank test, and used the graphical mode-shift method (Luikart *et al*. 1998) to qualitatively assess signatures of a bottleneck for each population.

Seven of the ten microsatellite loci used here were identified as having significant heterozygosity deficits in the original primer description, and the authors hypothesized that null alleles were the cause of this departure from HWE (Zakharov & Hellman 2007). To investigate potential null alleles, we used MICRO-CHECKER (Van Oosterhout 2004) and FreeNA (Chapuis & Estoup 2007) with default parameters to test for their presence. Both of these methods estimate the presence of null alleles based on the distribution of excess homozygosity among alleles; because hybrid systems and datasets containing multiple species may be expected to exhibit non-equilibrium homozygosity, we tested for null alleles within populations (geographic collection localities) and within broad regions that were delimited by species (e.g. all populations of *P. m. dodi* in the Drumheller area). We also estimated F_{ST} corrected for the inclusion of null alleles, using 1000 bootstrap replicates in FreeNA, and compared corrected to uncorrected F_{ST} values with a Wilcoxon rank sign test, conducted in R v3.1.1 (R Development Core Team).

4.4.4 Microsatellite genetic clustering

We used two methods to assess population structure: discriminant analysis of principal components (DAPC: Jombart *et al.* 2010) and Bayesian cluster analysis in STRUCTURE (Pritchard *et al.* 2000). We conducted DAPC in R v3.1.1 using the package *adegenet* v1.4.2 (Jombart 2008). The *find.clusters* function was used to identify the ideal *k* value, and *xvalDapc* was used sequentially using 100 replicates, to determine the optimal number of principal components to retain in the discriminant analysis (first with 200 then 50 maximum principal components, ultimately predicting 25 principal components). STRUCTURE was run using an admixture model and independent allele frequencies, a burn-in period of 150,000 MCMC generations followed by 500,000 generations for *k* values from one to ten, with 10 iterations per *k* value. The Evanno method (Evanno *et al.* 2005) was implemented with the full search method of STRUCTURE HARVESTER (Earl and vonHoldt 2012) to evaluate Δ*k*, and thus the most likely number of genetic clusters, and CLUMPP v1.1.2 (Jakobson & Rosenberg 2007) was used to average replicate runs. STRUCTURE was also run using population origin of individuals as a prior, but this did not alter overall patterns of genetic clustering (not shown), so we focus on results without this prior.

4.4.5 Hybrid classification

To better determine if the microsatellite markers used here are effective for detecting and classifying hybrids (F1, F2, backcross, etc.), we simulated five datasets derived from our microsatellite genotype data using HybridLab (Nielsen *et al.* 2006). By creating simulated datasets with individuals of known parentage, we can quantify the statistical power of these markers for classifying hybrids in an ideal situation (Vähä & Primmer 2006, Cullingham *et al.* 2011). To begin, we used the $k = 2$ results from STRUCTURE to identify “pure” parental individuals that exhibited ancestry > 0.99 for both *P. machaon* and *P. zelicaon*. From these “pure” individuals, we selected 40 individuals from multiple “typical” localities for each species (i.e. excluding individuals from the Swan Hills and the Foothills regions) to represent the genetic characteristics of each parent; these were used as input to simulate 50 profiles for each parental species. From these simulated parental individuals, we then simulated 50 individuals for each of the following hybrid classes: F1, F2, back-cross to *P. machaon* (i.e. an F1 mated to *P. machaon*: BXM), and back-cross to *P. zelicaon* (BXZ). Because of the distinct characteristics of *P. m. dodi* in the Red Deer River and *P. m. pikei* in the Peace River regions (see Results), we simulated data for these regions separately (using *P. zelicaon* from each respective region). This simulation regime resulted in five datasets consisting of 600 individuals each (300 individuals per region per dataset). To avoid biasing results due to the unique characteristics of each region, analyses on all simulated datasets were run separately for each region, but are combined for presentation of the results.

We used several methods to identify and classify hybrid individuals in the simulated and empirical datasets. First, we conducted clustering analysis in STRUCTURE, with identical settings and priors as above. The ancestry estimates produced by STRUCTURE (Q when $k = 2$) are akin to a hybrid index, where values of zero and one correspond to “pure” parent species, and intermediate values to hybrids (e.g. an F1 would be expected to have a score of 0.5). To allow for a degree of variability in the data, we used a Q threshold of ≥ 0.9 to assign individuals to each parental species (Cullingham *et al.* 2011).

We also explicitly classified individuals as hybrids using Bayesian assignment implemented in NewHybrids v1.1 (Anderson & Thompson 2002). This method generates posterior probabilities that individuals belong to one of six classes, “pure” *P. machaon* or *P. zelicaon*, an F1 or F2 hybrid, or a backcross resulting from an F1 hybrid mating with a “pure” parent of *P. machaon* or *P. zelicaon*. All analyses using NewHybrids were run with 250,000 burn-in generations, 500,000 generations, Jeffreys-like priors (Gelman 1996), and no allele frequency priors. NewHybrids was run with and without considering known parental individuals as a prior. The overall results did not change with the addition of this prior (not shown), so we focus on the results obtained without the additional prior. A single replicate of NewHybrids was run for each simulated dataset, and five replicates were averaged for the empirical dataset.

Classification of hybrids into distinct categories based solely on ancestry is convenient, but may over-simplify the complex nature of hybridization (Fitzpatrick 2012). To address this issue, we visualized the joint distribution of ancestry and interspecific heterozygosity using default methods in the package *introgress* (Gompert & Buerkle 2009, 2010) in R v3.1.1. This method uses parental allele frequencies to inform the estimation of a hybrid index for admixed individuals, and plots this relative to interspecific heterozygosity. We combined our five simulated datasets for this analysis, creating a large dataset with 250 parents per species per region (1000 total: considered "parental" individuals in the hybrid index estimation), and 250 individuals per hybrid class per region (2000 total: considered "admixed" individuals in the hybrid index estimation). Analysis of this simulated dataset was used to estimate 95% confidence intervals for each hybrid class in the joint distribution of ancestry and heterozygosity. Confidence intervals were created using the *car* package (Fox & Weisberg 2011) in R v3.1.1. These confidence intervals describe the approximate placement of each hybrid class in this distribution, and were then used to interpret the results of identical analysis with our empirical data. For the empirical data, all individuals that exhibited ancestry > 0.99 (in STRUCTURE for $k = 2$) for either the *P. machaon* or *P. zelicaon* cluster were considered "parental" individuals of their respective species, and the remaining individuals were considered "admixed". This resulted in 190 *P. machaon*, 311 *P. zelicaon*, and 280 "admixed" individuals.

4.4.6 Genome-wide SNPs

Genome-wide data can provide a more detailed assessment of population structure and ancestry than is possible with a small number of microsatellite markers (e.g. Helyar *et al.* 2011 *and references within*). Here we used a genotyping-by-sequencing approach (Poland *et al.* 2012) to generate genome-wide SNPs for 53 individuals: Nine *P. m. dodi*, ten *P. m. pikei*, seven *P. zelicaon*, and 27 putative hybrids from the Foothills region. Each species/subspecies included individuals from multiple localities, although low sample sizes limited realistic population genetic inferences. DNA extraction followed the aforementioned methods but was carried out with thoracic tissue, and included bovine pancreatic ribonuclease A (RNase A, Sigma) treatment and a subsequent ethanol precipitation. 96-plexed GBS libraries were prepared as in Poland *et al.* (2012) with the *PstI-MspI* restriction enzyme pair by the Institut de biologie intégratives et des systèmes (IBIS) at the Université Laval (Quebec City, QC). Pooled, adapter-ligated libraries were normalized with a duplex-specific nuclease treatment (Zhulidov *et al.* 2004), and further selectively amplified with a reverse primer containing an additional base (C) to selectively amplify one-quarter of the total amplified reads (Sonah *et al*. 2013). Single-end sequencing of 100 base-pair reads was conducted in three lanes of an Illumina HiSeq2000 at the McGill University-Génome Québec Innovation Centre (Montral, QC).

We analyzed FASTQ formatted data with publicly available software and default parameters, unless mentioned below. The *process_radtags* component of Stacks (Catchen *et al.* 2011, Catchen *et al.* 2013) was used to demultiplex and filter raw reads; final sequences were truncated to 92 bp to accommodate the maximum barcode length of eight base pairs. We then used a custom perl script to identify adapter sequence using a regular expression search and several utilities in the BioPerl toolkit (Stajich *et al.* 2002). In this script, reads were queried for the full adapter sequence (64 bp: Poland *et al.* 2012) followed by any portion of the 5' end of the adapter to a minimum of 16 bp. Adapter sequence is then trimmed, as well as five additional bp to account for possible sequencing error at the 3' end of each read. If the resulting sequence was < 32 bp in length, it was removed. We used BWA v0.7.12 (Li & Durbin 2009) to align our reads to 10,777 scaffolds of the *P. xuthus* reference genome (NCBI: GCA_000836235.1, accessed 17 March 2015), and then used the *pstacks*, *cstacks*, and *sstacks* components of Stacks to identify variants and determine genotypes for individuals. Finally, the *populations* component of Stacks was used to create output files, using the following parameters: minimum percent of individuals to process a locus (-r) = one; minimum number of populations containing each locus (-p) = one; minimum stack depth (-m) = four; and a minor allele frequency (-a) = 0.05. vcftools (Danecek *et al.* 2011) was used to remove non-biallelic SNPs and loci with > 5% missing data per individual, and data formats were converted manually and using PGD Spider (Lischer & Excoffier 2012). These bioinformatic steps were carried out in five custom perl wrappers, which are available with documentation at https://github.com/muirheadk/GBS_analysis_pipeline.

Two SNP datasets were generated: one including all SNPs passing the aforementioned filtering steps, and one with only parentally informative loci. The latter were defined as loci where either unique alleles were fixed for *P. zelicaon* or *P. machaon*, or where the frequency difference between the two species' alleles was greater than the 95% percentile of the frequency difference across all loci. Basic population genetic statistics were calculated as with the microsatellite data. To compare the capacity of these SNP datasets to identify and classify hybrid individuals, simulated datasets were created with the parentally informative loci, as with the microsatellite dataset. Here, we identified "pure" parental individuals by their geographic location, morphology, and the mtDNA and microsatellite data analyses. We simulated separate datasets for *P. m. dodi* and *P. m. pikei*; however, due to the low numbers of pure *P. zelicaon*, we used all of the available *P. zelicaon* individuals for both simulated datasets (including two individuals from the Peace River and five from the Red Deer River regions). One individual collected from a typical *P. m. pikei* locality exhibited hybrid *zelicaon*-like characteristics and was treated as a hybrid for all analytical purposes; we refer to this individual as the "*P. m. pikei* hybrid". Only 20 individuals of each hybrid class were simulated, due to the limited number of parental individuals. Analyses in STRUCTURE, DAPC, NewHybrids, and Introgress were also conducted as with the microsatellite dataset. NewHybrids was only used on the parentally informative dataset (due to program limitations when using large numbers of markers) with 50,000 burn-in generations and 200,000 generations. NewHybrids analysis was conducted on the empirical dataset separately for *P. m. dodi* and *P. m. pikei* (as was done with microsatellites), but also as a combined analysis (with all individuals) due to the limited number of *P. zelicaon*; results did not differ substantially between these analyses, so we present the latter here.

4.4 Results

4.4.1 mtDNA

After trimming regions of variable missing data from alignment margins, the 594 bp alignment had 34 parsimony-informative characters. Gene trees constructed with MP and BI had identical topologies, except for the presence of one tritomy in the MP tree that was resolved in the BI tree (Figure 4.2). The topology of *P. machaon*, *P. zelicaon* and the hybrid clade matched that of Dupuis & Sperling (2015). Most hybrid haplotypes were found in the foothills region in southwest AB, although one individual from the Swan Hills and one from the Peace River valley also displayed hybrid haplotypes (Figure 4.2). Haplotypes matching *P. machaon* and *P. zelicaon* were found in varying proportions in the Foothills region, while in the Swan Hills no *machaon*-like haplotypes were found. In the Red Deer and Peace River valleys, most *machaon*-like haplotypes were found at localities in steep river valleys or on bank edges, while most *zelicaon*-like haplotypes were found on isolated hilltops away from river valleys. The exceptions to this trend were several individuals of *P. m. dodi* in the Red Deer River valley that were found at typical *P. zelicaon* localities, and presumably were dispersers from nearby *P. m. dodi* habitat. Similar putative *P. zelicaon* dispersers were observed in the Peace River valley, as well as one *machaon*-like haplotype found at a typical *P. zelicaon* locality (Figure 4.2).

4.4.2 Microsatellite data properties

Genotype data for ten loci and 781 individuals gave an average of 24.6 alleles per locus (Table 4.1). Observed heterozygosity, expected heterozygosity, and the inbreeding coefficient ranged from 0.371 to 0.780, 0.473 to 0.891, and 0.101 to 0.325, respectively, across populations. Regions exhibited similar, but less variable, values for all measurements. Most pair wise F_{ST} comparisons between local populations were significant (424 out of 703 comparisons: Appendix 4.2), with differentiation between populations generally matching patterns of differentiation between regions (Table 4.2). Among regional comparisons, the Foothills and Swan Hills were less differentiated from regional samples containing predominately *P. zelicaon* (F_{ST} ranging from 0.003 to 0.026) than from regions containing *P. machaon* (ranging from 0.124 to 0.210). Comparisons of regional samples containing predominately *P. machaon* to those containing predominately *P. zelicaon* also resulted in moderate to high levels of differentiation (ranging from 0.104 to 0.231). Measures of gene diversity among individuals in regions were largely congruent among areas containing *P. zelicaon* and those in the Foothills and the Swan Hills

(Table 4.1); however, *P. machaon* exhibited relatively lower diversity, particularly *P. m. pikei* in the Peace River region. Using the Wilcoxon sign rank test and two models of microsatellite mutation, there were no significant signatures of a recent population bottleneck in any locality, although 17 of 38 localities exhibited signatures of recent population expansions (Appendix 4.3). Four localities qualitatively displayed a signature of a recent bottleneck with the mode-shift method, but each of these had sample sizes less than ten individuals.

4.4.3 Null alleles

Only five of 38 local populations displayed genotypic proportions expected under HWE (Table 4.1), and no loci were consistent with HWE when all populations were averaged. MICRO-CHECKER detected null alleles in an average of 3.657 loci per population (ranging from one to eight loci in local populations; Appendix 4.4). When regions and species were considered, the average number of loci with null alleles increased to 7.5 and 9.5, respectively, with values ranging from six to nine loci in regions and nine to ten loci in species. MICRO-CHECKER (using Oosterhout estimates) and FreeNA produced similar estimates of the frequencies of null alleles per locus, with averages across all populations equaling 0.076 and 0.082, respectively. For regions, the average estimated frequency of null alleles per locus increased to 0.098 and 0.092 for MICRO-CHECKER and FreeNA, respectively. Pairwise F_{ST} values corrected for null alleles were consistently lower than uncorrected values (mean difference = 0.0072, Appendix 4.2), as is expected (Chapuis *et al.* 2008), but these estimates did not differ significantly (Wilcoxon rank sign test, W = 239667.5, $p = 0.3286$).

Traditional population genetics workflows generally suggest the exclusion of loci exhibiting excess homozygosity due to null alleles (e.g. Allendorf & Luikart 2007). However, such departure from HWE can also be the result of biological processes (mating preferences or demographic history, or cryptic population structure, e.g. Wahlund effect). Although the locus-specificity of null alleles is often cited as a way to distinguish them from biological processes (e.g. Dakin & Avise 2004, DeWoody *et al.* 2006), this is not always a straightforward task (Dharmarajan *et al.* 2013). This is especially true in systems for which we lack a thorough understanding of effective population size, number of demes, and migration rate/dispersal between populations (Dharmarajan *et al.* 2013), all of which are poorly known in the *P. machaon* complex. Additionally, microsatellites are known to be difficult to develop for Lepidoptera (Ji *et al.* 2003, Meglécz *et al.* 2004, Zhang 2004), and the fact that these microsatellites were developed only for *P. zelicaon* (Zakharov & Hellman 2007) is likely to increase the probability of null alleles for *P. machaon* (Li *et al.* 2003, Chapuis & Estoup 2007). Most importantly, we are studying an area of secondary contact between divergent species and thus do not expect HWE. For these reasons, we did not exclude any loci from subsequent analysis. However, due to the potential influence of null alleles on fine-scale population structure we have limited our focus to broader evaluations of population structure, which are generally affected less by the presence of null alleles (Dakin & Avise 2004, Chapuis & Estoup 2007, Dharmarahan *et al.* 2013).

4.4.4 Microsatellite genetic clustering

Using the *find.clusters* technique, DAPC analysis of the microsatellite data predicted the ideal number of genetic clusters (k) to be ten; however, visualization of these clusters clearly supports three main groupings corresponding to *P. m. dodi*, *P. m. pikei*, and a third group containing *P. zelicaon* and individuals from the Swan Hills and the Foothills regions (Figure 4.3a). Analysis in STRUCTURE produced virtually identical results, with $k = 2$ and $k = 3$ being highly supported (Δk for $k = 2$ to 9 equals 3554.6, 1193.1, 57.1, 0.7, 4.9, 0.7, 12.5, and 1.0 respectively). Visually, $k = 2$ separated *P. machaon* from *P. zelicaon* and $k = 3$ further separated the *P. machaon* group into subspecific clusters (Figure 4.4a-b).

The taxonomic identity of the main clusters from both microsatellite analyses was confirmed by clear congruence with mtDNA clusters. Regression analysis using a linear model on the Q-values from STRUCTURE compared to the mtDNA clade of each individual showed significant correlation between nuclear ancestry and mtDNA clade ($F_{1,269} = 475.5$, $R^2 = 0.6387$, $p < 0.00001$; individuals in *P. machaon* or *P. zelicaon* clades were assigned values of one and two, and those exhibiting hybrid mtDNA excluded). Both DAPC and STRUCTURE analyses also identify several individuals that appear to be dispersers, and the majority of these are the same individuals identified as dispersers with mtDNA (*P. m. dodi* individuals collected at typical *P. zelicaon* localities in the Red Deer River region, and *P. zelicaon* collected at typical *P. m. pikei* localities in the Peace River region; Figures 4.3, 4.4). Additionally, several intermediate individuals, indicative of early generation hybrids, can be identified in both analyses but particularly in the ancestry estimations of the STRUCTURE results (see below, Hybrid classification). Most of these individuals were collected from the Red Deer River valley and the Foothills regions, with very few originating from the Swan Hills, and only one intermediate individual from the Peace River valley.

4.4.5 Microsatellite hybrid classification

Analysis of our simulated datasets showed that the microsatellite markers used here potentially differentiate parental and hybrid individuals well. Average identification success (Q or probability ≥ 0.9 in STRUCTURE and NewHybrids, respectively) of simulated individuals to

P. machaon, *P. zelicaon*, or a hybrid class ranged from 90.4 to 95.8% for STRUCTURE and 74.2 to 93.1% for NewHybrids, respectively (Table 4.3, single simulation for each analysis shown in Figure 4.5, all simulations shown in Appendices 4.5-4.6). Differentiating hybrid classes in NewHybrids was less successful, however qualitative assessment (Appendix 4.6) indicates that most hybrid misclassification was due to hybrid individuals being assigned to multiple hybrid classes. Qualitatively, hybrid classification was more successful for F1 hybrids and backcrosses than for F2s (Appendix 4.6).

Applying similar Q thresholds (hybrid between 0.1 and 0.9) to the empirical dataset, STRUCTURE and averaged NewHybrids results (the average standard deviation of assignment probabilities across 5 replicates was 8.057×10^{-5}) predicted similar numbers of parents and hybrids (Table 4.3, Figure 4.4). However, only nine individuals in the NewHybrids results had a combined hybrid probability > 0.9, compared to 45 with STRUCTURE. Most remaining individuals had mixed probabilities between hybrid and parental classes. No individuals were predicted to be F1s or back-crosses to *P. zelicaon* (BXZ) with high probability. Only one individual had an F1 probability > 0.01 (specifically 0.01001) and only 16 had a BXZ probability > 0.01 (average BXZ probability across individuals was 0.001, and the maximum probability observed was 0.084). For this reason, those hybrid classes are omitted from Figure 4.4c.

The parental allele-informed hybrid index produced by *introgress* for our empirical dataset generally matched that predicted by STRUCTURE, which is not informed by parental characteristics (Figure 4.6a). Most admixed individuals exhibited parentally skewed ancestry: Individuals from the Foothills and Swan Hills displayed *P. zelicaon*-like ancestry, while those from typical *P. machaon* localities were skewed towards that parent. Considering the joint distribution of ancestry and interspecific heterozygosity, and the 95% confidence intervals created from our simulated datasets, we observed large overlap between hybrid classes (Figure 4.6a), indicating relatively low power in distinguishing classes of hybrid individuals with this method. If we only consider individuals located in non-overlapping regions of these confidence intervals, 138 hybrids are classified (Table 4.3).

4.4.6 Genome-wide SNPs

A total of 127 million reads contained unambiguous barcodes allowing specimen identifications (an average of 2.4 million reads per individual), and after initial quality control 101 million reads remained (average per individual: 1.9 million). Of these, 21 million reads mapped to the *P. xuthus* genome (average per individual: 0.4 million), which resulted in 7,606 loci being identified, with an average read depth across individuals of 108.3 reads (minimum and maximum per individual read depth equaled 32.6 and 255.9 reads, respectively). General filtering (see methods) and filtering for parentally informative loci resulted in 1,614 and 37 SNPs, respectively. Because of the limitations of the number of individuals sampled for the SNP dataset, descriptive statistics were calculated separately for each species (subspecies for *P. machaon*) and for the individuals from the Foothills region, rather than for each geographic population (Tables 4.3, 4.4). The overall pattern of these results matched that of the microsatellite dataset, with lower heterozygosity and genetic diversity in *P. m. pikei* in the Peace River valley, higher heterozygosity and diversity in the Foothills region, and otherwise relatively comparable values between regions. Most species/regions did not display HWE genotypic proportions (Table 4.4), as would be expected with hybridization. Parentally informative SNPs produced higher estimates of pair wise F_{ST}, and all pair wise comparisons showed significant differentiation, except those comparing *P. zelicaon* to individuals from the Foothills region

(Table 4.5).

DAPC of the full (1614 loci) SNP dataset predicted three clusters, and clearly demarcated *P. m. dodi*, *P. m. pikei*, and *P. zelicaon* and the putative hybrids (Figure 4.3b). Analysis with STRUCTURE supported $k = 2$ and $k = 4$ (Δk for $k = 2$ to 9 equals 1291.9, 181.7, 335.7, 0.6, 13.2, 1.0, 6.3, and 0.7 respectively); while $k = 2$ separated *P. machaon* from *P. zelicaon* and putative hybrids, *P. m. dodi* individuals have intermediate Q values resembling hybrids (Figure 4.7a). At $k = 4$, *P. m. dodi* formed a unique cluster, and putative hybrids from the Foothills region were split between a *zelicaon*-like cluster and a unique hybrid cluster (Figure 4.7a). When parentally informative SNPs were analyzed, DAPC predicted three clusters as with the larger SNP dataset, but less separation was apparent between *P. m. dodi* and *P. m. pikei* (Figure 4.3c). STRUCTURE supported $k = 2$ and $k = 3$ (Δk for $k = 2$ to 9 equals 6237.6, 154.4, 18.5, 1.5, 0.5, 1.1, 0.9, and 1.0 respectively) with parentally informative SNPs. Clustering at $k = 2$ was similar to that with the larger SNP dataset, but *P. m. dodi* was less ambiguous; at $k = 3$, individuals from the Foothills were split between a *zelicaon*-like cluster and a unique cluster (Figure 4.7b).

4.4.7 SNP hybrid classification

The parentally informative SNPs were highly successful at identifying simulated parental and hybrid individuals. Average identification success of simulated individuals to *P. machaon*, *P. zelicaon*, or a hybrid class was much higher than with microsatellites, as was identification of specific hybrid classes in NewHybrids (Table 4.3, Appendices 4.7-4.8). In the empirical SNP dataset, parental individuals were chosen explicitly, and using a Q threshold of 0.9 from the parentally informative $k = 2$ results from STRUCTURE, all parents except four of the *P. m. dodi* were identified as parents correctly (decreasing the classification threshold to 0.8 correctly identifies all parents). Putative hybrids were classified either as hybrids or as *P. zelicaon* (Figure 4.7b). Averaged NewHybrids results (average standard deviation between replicates = 1.025 x 10^{-4}) for the empirical, parentally informative SNP dataset correctly identified all parental individuals with probability higher than 0.9 (Table 4.3, Figure 4.7c). The putative hybrids we generally assigned to either *P. zelicaon* or F2 hybrid class, matching the split observed in STRUCTURE with both parentally informative SNPs and all SNPs. No individuals had a probability of BXM > 0.01, so this class is omitted from Figure 4.7c; the only individual with a probability of F1 > 0.0 was the *P. m. pikei* hybrid individual (indicated in Figure 4.7c). The joint distribution of heterozygosity and hybrid index estimated by *Introgress* largely matched the results from STRUCTURE and NewHybrids. The 95% confidence intervals created with the combined simulated datasets overlapped much less than with the microsatellite datasets, but the majority of empirical individuals still fell outside of these confidence intervals (22 of 28 individuals) (Figure 4.6b). Regardless, two main clusters of individuals were observed: One resembling F2 hybrids, and one resembling BXZ hybrids. The *P. m. pikei* hybrid individual was the only individual that did not cluster with the main groups, and was placed between the F1 and F2 confidence intervals.

4.5 Discussion

Identification and classification of hybrid individuals can have major ramifications for taxonomic interpretation, phylogenetic reconstruction, and conservation management. However, the task is far from simple in systems with complex evolutionary histories and variable hybrid signatures. The *P. machaon* species complex is a prime example of evolutionary complexity at multiple spatial and temporal scales: Hybrid lineages with distinct evolutionary backgrounds, but similar retained parental signatures, are found across North America (Dupuis and Sperling 2015). In the present study, we focused on a zone of hybrid interaction between *P. machaon* and *P. zelicaon* in western Canada and ask whether multiple genetic and genomic datasets provide a similar interpretation of the hybrid situation to that found previously with morphology and allozymes. In doing so, we also compared the success of multiple methods of hybrid identification and classification across these datasets.

4.5.1 Population structure

Overall population structure across the study area was dominated by the interspecific differentiation between *P. machaon* and *P. zelicaon*, corroborating previous results (Sperling 1987). Across most of the study area mtDNA displayed a clear demarcation between typical *P. machaon* habitat in arid river valleys, and typical *P. zelicaon* habitat on prairie and forest hilltops (Figure 4.2). In the Foothills region of Alberta, where most intermediate individuals were observed, there were variable mtDNA clade frequencies, as well as higher frequencies of the unique hybrid mtDNA. These unique haplotypes are present across North America in hybrid lineages, and may represent the influence of an ancestral lineage of *P. machaon*, likely similar to *P. m. hudsonianus* (Dupuis & Sperling 2015). On the whole, microsatellites and SNPs displayed the same pattern as mtDNA by separating *P. machaon* from *P. zelicaon*, although the status of *P. m. dodi* is more complex (discussed below). Despite high levels of mitochondrial polymorphism found in *P. machaon* (present study, Dupuis & Sperling 2015), nuclear loci distinguished the subspecies of *P. machaon* regardless of the analysis (Figure 4.3, Figure 4.4), which supports their evolutionary distinctness (Sperling 1987, Bromilow & Sperling 2011).

Individuals in both the Swan Hills and Foothills regions had predominately *zelicaon*-like ancestry based on microsatellite markers (Figure 4.3a, Figure 4.4). Although admixed individuals were observed (especially in the Foothills region), the overall frequency of hybrids was much lower than that found in previous research using allozymes and morphological characters. Sperling (1987) concluded that almost all of the individuals in some localities of the Foothills region were hybrids; indeed, conducting Bayesian clustering analysis in STRUCTURE using the electrophoretic dataset of Sperling (1987) (with identical methods as in the present study) leads to a similar conclusion (Appendix 4.9). This discrepancy could be explained by functional differences between the datasets; microsatellites are presumably neutral, while allozymes are more likely to be under selection, and the effect of this difference on estimates of population differentiation is widely documented in the literature (e.g. Allendorf & Seeb 2000, De Innocentiis *et al*. 2001, Larsson *et al.* 2007). The parentally informative SNP dataset may also represent loci that are adaptive, as these are markers for genomic regions that may support the maintenance of the species boundary between *P. machaon* and *P. zelicaon* (Payseur 2010).

For the admixed individuals observed in our dataset, there was no apparent trend relating the unique hybrid-type mtDNA haplotypes to admixed microsatellite ancestry; only three individuals in the Foothills region with hybrid mtDNA had Q values between 0.1 and 0.9 in the $k = 2$ STRUCTURE results. Ancestry estimates with the SNP datasets displayed two different groups of individuals in the Foothills region, one matching *P. zelicaon*, and the other forming a distinct hybrid-like cluster (Figure 4.7). Individuals belonging to this distinct cluster had more variable mtDNA (Figure 4.7d), and potentially a higher prevalence of admixture with microsatellite markers (Figure 4.7e). However, the limited sampling of these SNP datasets may have biased STRUCTURE results (Shringarpure & Xing 2014), and we observe very little separation of Foothills region individuals with DAPC (Figure 4.3b-c). Regardless, the SNPs appear to show some nuclear similarity between these hybrids and *P. m. dodi*, which may explain the high frequency of more typical *machaon*-like mtDNA haplotypes (i.e. non hybrid haplotypes) in the region.

An alternative explanation for the discrepancy between our results and those of Sperling (1987) is that the hybrid interaction has changed in the last 30 years. While this is a possibility, the overall appearance of intermediate individuals in this region is not noticeably different than in the 1980's (Dupuis & Sperling, *personal observations*). Furthermore, similar intermediate individuals exist from the early 1900's (Sperling 1987), supporting the hypothesis that this is a stable hybrid situation. Without comparable temporal datasets that are genotyped with the same set of markers (e.g. Smith *et al.* 2013), hypotheses about the status of this hybrid interaction are anecdotal. Additionally, the mosaic pattern of this interaction (Harrison & Rand 1989) makes it more difficult to track changes, as compared to a linear or tension hybrid zone (Barton & Hewitt 1985). Although more cases of moving hybrid zones are being reported (Buggs 2007), many documented cases of stable hybrid zones also exist (e.g. Lukhtanov *et al.* 2005, Nosil & Yukilevich 2008, Latch *et al.* 2011, Smith *et al*. 2013), and these results underline the importance of caution in interpreting data from single (mtDNA) or few (allozyme or microsatellite) loci in the context of hybridization.

The clear discordance between nuclear and mitochondrial genomes in this region suggests a complex and lengthy history of hybridization. With introgression from either *P. m. hudsonianus* or *P. m. dodi* into a background of *P. zelicaon* at potentially very different temporal scales, the situation in the *P. machaon* species complex may be akin to that observed in *Lycaedes* butterflies in western North America. In these lycaenids, widespread historical and recent admixture has created patterns of genetic variability that complicate interpretation of hybrid lineages (Gompert *et al.* 2014). Some ecological influences on *Lycaedes* hybridization have been documented (Gompert *et al.* 2006), whereas this information is lacking in the *P. machaon* complex and would likely provide great insight. Even if potentially different temporal scales are ignored, hybridization by multiple parental subspecific taxa could translate to inter- and intraspecific variability in reproductive isolation, leading to diverse outcomes for hybridization at both genomic and ecological scales (Teeter *et al.* 2010, McDermott & Noor 2011, Cutter 2012, Gompert *et al.* 2014, Larson *et al.* 2014). For instance, hybridization between different pairs of *Catostomus* fishes results in heterogeneous outcomes, depending on the species pairs involved and their geographic origin (Mandeville *et al.* 2015). Clearly, more ecological work is needed in the *P. machaon* complex.

4.5.2 Unexpected hybrid signatures

The anticipated complexity of the hybrid situation in the Foothills region was, unexpectedly, matched by complexity of *P. m. dodi* in the Red Deer River region. None of the individuals collected at typical *P. m. dodi* localities displayed morphological signs of intermediacy, and mtDNA mimicked this pattern. However, microsatellites revealed substantial admixture (Figures 4.4a,b) and late generation hybrid signatures (Figure 4.4c) in many individuals, while both SNP datasets resulted in unique signatures (Figures 4.3a,b, 4.7a,b). The presence of dispersing *P. m. dodi* at typical *P. zelicaon* habitats south of the Red Deer River valley indicate high vagility by *P. m. dodi*. One explanation for the observed hybrid influence is movement of vagile *P. m. dodi* to typical *P. zelicaon* hilltops, and subsequent hybridization of female *P. m. dodi* and male *P. zelicaon*. *Artemesia dracunculus*, the host of *P. m. dodi*, is only found in arid river valleys, so the oviposition requirements of these female butterflies should draw them back to typical *P. m. dodi* habitat. Given our microsatellite results, these events are likely rare enough so that the main hybrid signature is that of later generation hybrids, rather than F1s, which is corroborated by our *Introgress* results (Figure 4.6a). The genome-wide SNP datasets also provide some support for the potential admixed nature of *P. m. dodi*; however, limited sample sizes make it difficult to separate potential signatures of admixture from unique signatures of *P. m. dodi* separation from *P. m. pikei* (e.g. in Figure 4.7a, admixed *P. m. dodi* may reflect differentiation from *P. m. pikei* evident at $k = 4$). Furthermore, if the parentally informative SNPs are indeed more adaptive in nature than the larger SNP dataset (Luikart *et al.* 2003), then any role of these SNPs in maintaining species boundaries between *P. machaon* and *P. zelicaon* may further complicate the interpretation of potential admixture in *P. m. dodi*. More thorough geographic sampling with genome-wide loci should provide insight into this situation.

The *P. m. pikei* hybrid was also unexpected (see Figure 4.7). This individual was collected at a typical *P. m. pikei* locality, exhibited *P. zelicaon*-like mtDNA, and had *zelicaon*-like and hybrid-like microsatellites and SNPs, respectively. Morphologically, this individual was intermediate between *P. m. pikei* and *P. zelicaon*, and to our knowledge is the only genetically documented hybrid individual between these taxa (Sperling 1987). While occasional hybrids are not surprising, given the propensity for hybridization in the species group, it provides an interesting contrast between two seemingly similar environments - the Peace River and Red Deer River regions. Both areas have the same host plants, and the habitats are seemingly used in the same way by *P. machaon*, living directly in the river valleys, and *P. zelicaon*, on the hilltops surrounding the river valleys. The rarity of hybridization in the Peace River valley may indicate different reproductive isolation in *P. m. dodi* versus *P. m. pikei*, as well as different origins.

4.5.3 Hybrid identification and classification

Given the complex hybrid interactions in this species group, the *P. machaon* species group provides a rich foundation from which to compare multiple hybrid identification and classification approaches. The methods used here have varied utility for identifying versus classifying hybrids. For instance, the joint distribution approach in *Introgress* is useful for classifying categories of hybrids, but relies on *a priori* identification of admixed individuals, so does not have utility by itself for identifying hybrids. Therefore, we consider these two objectives separately, and compare hybrid identification in STRUCTURE and NewHybrids, and hybrid classification in NewHybrids and *Introgress*.

First, through the use of our simulated datasets, it is clear that our parentally informative SNP dataset, despite being comprised of only 37 biallelic loci, was more successful at identifying and classifying hybrids than our microsatellite dataset of 10 loci, which had a mean of 24.6 alleles per locus. While both datasets identified hybrid versus parental individuals relatively well, the microsatellite dataset did not effectively classify certain hybrid classes (e.g. F2 hybrids), while the SNP dataset was more successful across the board. This pattern was also apparent in the joint distribution approach of *Introgress*, where the overlap of 95% confidence intervals from microsatellites was much larger than that of SNPs. This result is expected, as having more loci should provide higher power for classifying hybrids, particularly when those markers are parentally informative; Fitzpatrick (2012) recommends at least 50 informative markers for confident classification of hybrid individuals. For our empirical datasets, the two Bayesian methods used here (STRUCTURE and NewHybrids) had relatively equal power for hybrid identification, corroborating previous findings (Cullingham *et al.* 2011). The two methods for hybrid classification (NewHybrids and *Introgress*), on the other hand, provide different interpretations of the hybridization in this system. While both methods identify more late- than early-generation hybrids, the joint distribution of heterozygosity and ancestry depicts a higher level of complexity in the system. This complexity can translate to less confident hybrid classification overall, but more accurately reflect the potential for temporal persistence of hybrids. Discrete hybrid classes can be misleading in systems that are beyond the first two generations of hybridization (Fitzpatrick 2012), which is certainly the case here (Sperling 1987). These results demonstrate the value of using multiple identification and classification strategies, since hybridization is potentially important in a variety of contexts (e.g. DeMarais *et al*. 1992, Haig *et al.* 2004, Monzón *et al.* 2014).

Table 4.1 Descriptive statistics for microsatellite data for localities, regions, and loci. Parenthetical codes after locality names indicate the region of each sample, and ".m" or ".z" indicate a separate species designation where regions are partitioned. RDR: Red Deer River region; FH: Foothills; SH: Swan Hills; PR: Peace River region; N: sample size or number of individuals genotyped per locus; Alleles: average number of alleles per locality or region, and total number of alleles per locus; H_O: observed heterozygosity; H_S: expected heterozygosity; G_{IS}: inbreeding coefficient (analogous to F_{IS}); HWE: p-values of G_{IS} testing for Hardy-Weinberg proportions (non-significant p-values after Bonferroni correction bolded); Div.: gene diversity among individuals or loci (1 - Qinter).

Locality, region or locus	N	Alleles	H_O	H_S	G_{IS}	HWE	Div.
Hand Hills (RDR.z)	10	9	0.724	0.891	0.187	<0.0001	0.890
Wintering Hills E (RDR.z)	32	14.9	0.666	0.868	0.234	<0.0001	0.868
Wintering Hills W (RDR.z)	24	13	0.703	0.868	0.190	<0.0001	0.867
Horsethief Canyon (RDR.m)	30	9.4	0.589	0.717	0.179	<0.0001	0.748
Lousana (RDR.m)	33	9.3	0.553	0.685	0.194	<0.0001	0.721
Morrin Bridge (RDR.m)	22	8.1	0.559	0.708	0.209	<0.0001	0.726
North Drumheller (RDR.m)	22	8.8	0.591	0.712	0.171	<0.0001	0.743
Orkney Lookout (RDR.m)	16	7.5	0.531	0.707	0.249	<0.0001	0.744
Tolman Bridge (RDR.m)	28	9.2	0.587	0.690	0.149	<0.0001	0.730
Antler Hill (FH)	15	9.7	0.741	0.849	0.126	<0.0001	0.850
Bragg Creek Ski Hill (FH)	14	8	0.564	0.824	0.315	<0.0001	0.824
Buck Mtn (FH)	82	14.5	0.647	0.829	0.220	<0.0001	0.829
Fish Butte (FH)	31	12.9	0.673	0.850	0.208	<0.0001	0.850
Jumpingpound Ridge (FH)	9	7.8	0.667	0.880	0.242	<0.0001	0.880
Medicine Hills (FH)	8	6	0.575	0.815	0.294	<0.0001	0.819
Mesa Butte (FH)	8	6.4	0.663	0.845	0.216	<0.0001	0.845
Powderface Ridge (FH)	8	7.7	0.763	0.875	0.129	**0.00417**	0.875
Shunda Mtn (FH)	13	9.2	0.655	0.849	0.229	<0.0001	0.848
Wildcat Hills (FH)	12	9.2	0.780	0.868	0.101	**0.00333**	0.868
Enilda Lookout (SH)	20	9.6	0.625	0.829	0.247	<0.0001	0.830
Goose Mtn (SH)	26	11.5	0.648	0.819	0.209	<0.0001	0.819
House Mtn (SH)	17	9.1	0.650	0.824	0.212	<0.0001	0.825
Marten Mtn (SH)	16	8.9	0.709	0.819	0.134	0.00014	0.818
Pushwaskau Lookout (SH)	18	9.1	0.659	0.820	0.197	<0.0001	0.819
Sweathouse Lookout (SH)	13	8.7	0.654	0.856	0.237	<0.0001	0.856
Whitecourt Mtn (SH)	14	8.4	0.714	0.843	0.153	<0.0001	0.844
Beatton River (PR.m)	13	3.4	0.400	0.473	0.154	**0.01349**	0.473
Clayhurst Ferry (PR.m)	7	3.2	0.500	0.556	0.101	**0.12424**	0.572
Highland Park (PR.m)	30	5.4	0.448	0.602	0.256	<0.0001	0.610

Kaufman Hill (PR.m)	22	5.8	0.484	0.584	0.172	<0.0001	0.592
Lynx Ridge (PR.m)	24	5.3	0.452	0.578	0.219	<0.0001	0.581
Shaftsbury Trail (PR.m)	22	4.1	0.444	0.517	0.141	**0.00234**	0.529
Taylor (PR.m)	31	4.4	0.371	0.488	0.239	<0.0001	0.490
Bear Mtn (PR.z)	26	10.1	0.637	0.795	0.200	<0.0001	0.794
Bullhead Mtn (PR.z)	15	8.6	0.670	0.833	0.195	<0.0001	0.836
Kleskun Hills (PR.z)	13	6.9	0.585	0.782	0.252	<0.0001	0.783
Saskatoon Mtn (PR.z)	24	9	0.671	0.806	0.167	<0.0001	0.806
White Mtn (PR.z)	13	7.2	0.543	0.805	0.325	<0.0001	0.801
all RDR *P. zelicaon* (RDR.z)	66	18.3	0.688	0.876	0.215	<0.0001	0.876
all RDR *P. machaon* (RDR.m)	151	13.8	0.570	0.703	0.189	<0.0001	0.737
all Foothills (FH)	200	19.5	0.663	0.852	0.222	<0.0001	0.852
all Swan Hills (SH)	124	15.2	0.661	0.831	0.205	<0.0001	0.831
all PR *P. machaon* (PR.m)	149	9.5	0.435	0.565	0.230	<0.0001	0.571
all PR *P. zelicaon* (PR.z)	91	13.9	0.628	0.808	0.223	<0.0001	0.808
locus D224c	763	23	0.613	0.662	0.074	<0.0001	0.652
locus A229T	764	21	0.529	0.768	0.312	<0.0001	0.760
locus B225T	758	29	0.509	0.820	0.380	<0.0001	0.821
locus B102T	762	29	0.602	0.856	0.296	<0.0001	0.849
locus B12T	666	28	0.406	0.632	0.358	<0.0001	0.668
locus A110	781	15	0.655	0.716	0.085	<0.0001	0.713
locus A117T	780	36	0.747	0.837	0.108	<0.0001	0.834
locus A121	724	23	0.544	0.773	0.297	<0.0001	0.773
locus A214	755	29	0.753	0.792	0.050	0.00093	0.781
locus B209T	776	13	0.720	0.765	0.058	0.00034	0.761

Table 4.2 Microsatellite pairwise F_{ST} comparisons for regions. Lower triangle: normal F_{ST}, bolded value indicates non-significant comparisons after Bonferroni correction ($p > 0.05$). Upper triangle: F_{ST} corrected for null alleles. RDR: Red Deer River region; PR: Peace River.

	1	2	3	4	5	6
RDR *P. zelicaon* (1)		0.103	0.020	0.024	0.230	0.026
RDR *P. machaon* (2)	0.104		0.094	0.117	0.165	0.124
Foothills (3)	0.021	0.124		0.005	0.184	0.011
Swan Hills (4)	0.026	0.146	0.005		0.203	0.003
PR *P. machaon* (5)	0.217	0.176	0.190	0.210		0.221
PR *P. zelicaon* (6)	0.030	0.156	0.012	**0.003**	0.231	

Table 4.3 Proportion of successful identification of simulated (Sim) parental and hybrid individuals, and classification results for empirical datasets (Emp). Classification of empirical data based on probabilities of assignment >0.9 (or for STRUCTURE results of hybrids $0.1 < Q < 0.9$). Hybrid refers to combined hybrid classifications when applicable, and individual hybrid classes are for unambiguous results only (individuals with split probabilities of multiple hybrid classes, or those that occur in overlapping confidence intervals for Introgress results, are excluded). MSAT: microsatellites, SNP: parentally informative SNPs, STR: STRUCTURE, NH: NewHybrids, INT: Introgress.

	P. machaon	*P. zelicaon*	hybrid	F1, F2, BXZ, BXM
Sim MSAT STR	0.904	0.946	0.958	NA
Sim MSAT NH	0.820	0.742	0.931	0.428, 0.280, 0.302, 0.320
Sim SNP STR	1.0	0.995	0.999	NA
Sim SNP NH	1.0	0.995	0.948	1.0, 0.935, 0.990, 0.970
Emp MSAT STR	285	451	45	NA
Emp MSAT NH	240	444	9	0, 6, 0, 0
Emp MSAT INT	NA	NA	NA	0, 30, 22, 86
Emp SNP STR	14	13	22	NA
Emp SNP NH	19	7	13	0, 13, 0, 0
Emp SNP INT	NA	NA	NA	0, 2, 4, 0

Table 4.4 Descriptive statistics for SNP datasets. All SNPs (1614 loci) before slash, and parentally informative SNPs (37 loci) after slash. H_O: observed heterozygosity; H_S: expected heterozygosity; G_{IS}: inbreeding coefficient (analogous to F_{IS}); HWE: *p*-values of G_{IS} testing for Hardy-Weinberg proportions (non-significant *p*-values after Bonferroni correction bolded); Div.: gene diversity among individuals (1 - Qinter).

	H_O	H_S	G_{IS}	HWE	Div.
P. m. dodi	0.128/0.228	0.143/0.260	0.108/0.122	<0.0001/**0.0316**	0.143/0.260
P. m. pikei	0.111/0.063	0.118/0.085	0.063/0.260	<0.0001/0.0068	0.117/0.085
Foothills	0.141/0.280	0.162/0.386	0.130/0.276	<0.0001/<0.0001	0.161/0.383
P. zelicaon	0.125/0.116	0.146/0.136	0.144/0.147	<0.0001/**0.0464**	0.151/0.214

Table 4.5 SNP pair wise F_{ST} comparisons for main species/region groupings. Lower triangle: All SNPs (1614 loci); upper triangle: parentally informative SNPs (37 loci). Bolded values indicate non-significant comparisons after Bonferroni correction ($p > 0.05$).

	P. m. dodi	*P. m. pikei*	Foothills	*P. zelicaon*
P. m. dodi		0.175	0.432	0.737
P. m. pikei	0.193		0.563	0.878
Foothills	0.105	0.221		**0.166**
P. zelicaon	0.167	0.329	**0.025**	

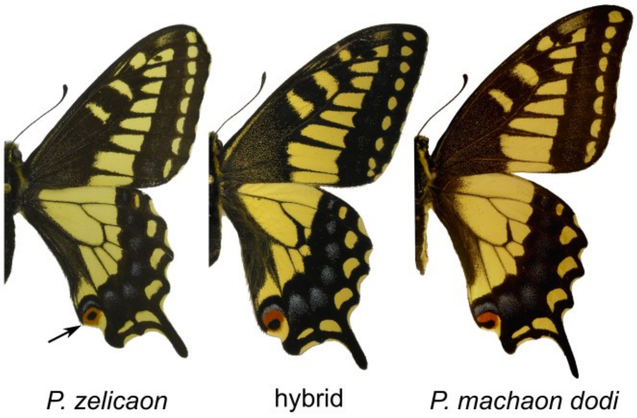

Figure 4.1 Right ventral habitus of a typical *P. zelicaon*, *P. machaon dodi*, and morphological intermediate individual (putative hybrid) from the Foothills region of Alberta. Arrow denotes eyespot on hindwing.

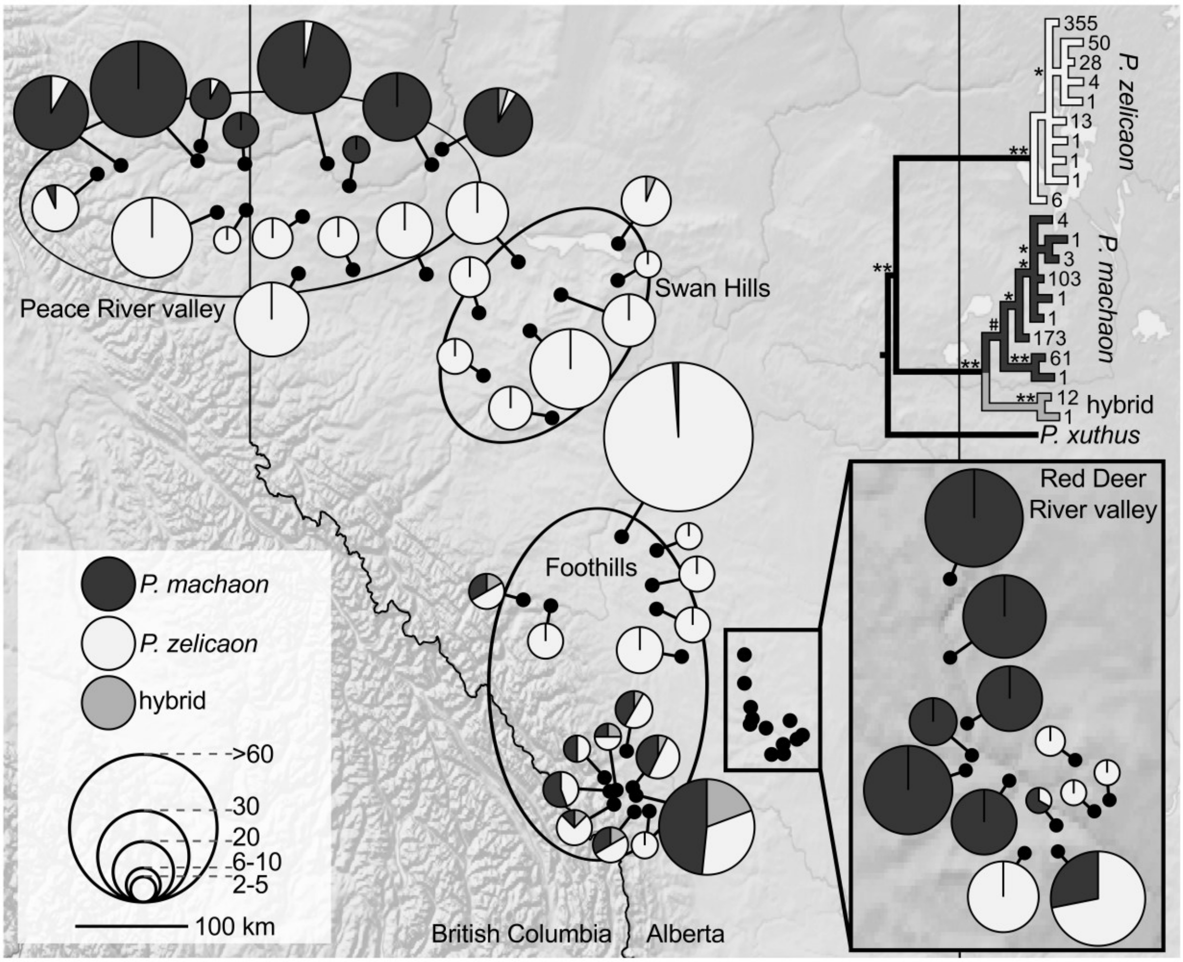

Figure 4.2 Mitochondrial DNA variation. Pie charts show proportions of *P. machaon*, *P. zelicaon*, and hybrid-type mtDNA haplotypes at each population. The size of the pie chart indicates sample size, and the inset tree gives a 50% majority rule consensus tree constructed using Bayesian Inference, with node labels indicating sample sizes per haplotype. Single and double asterisks to the left of nodes on inset tree correspond to > 80% bootstrap support in Bayesian inference only, and both Maximum Parsimony and Bayesian inference, respectively. The pound sign (#) on tree inset denotes the node that was not resolved consistently (< 50%) in Maximum Parsimony reconstruction.

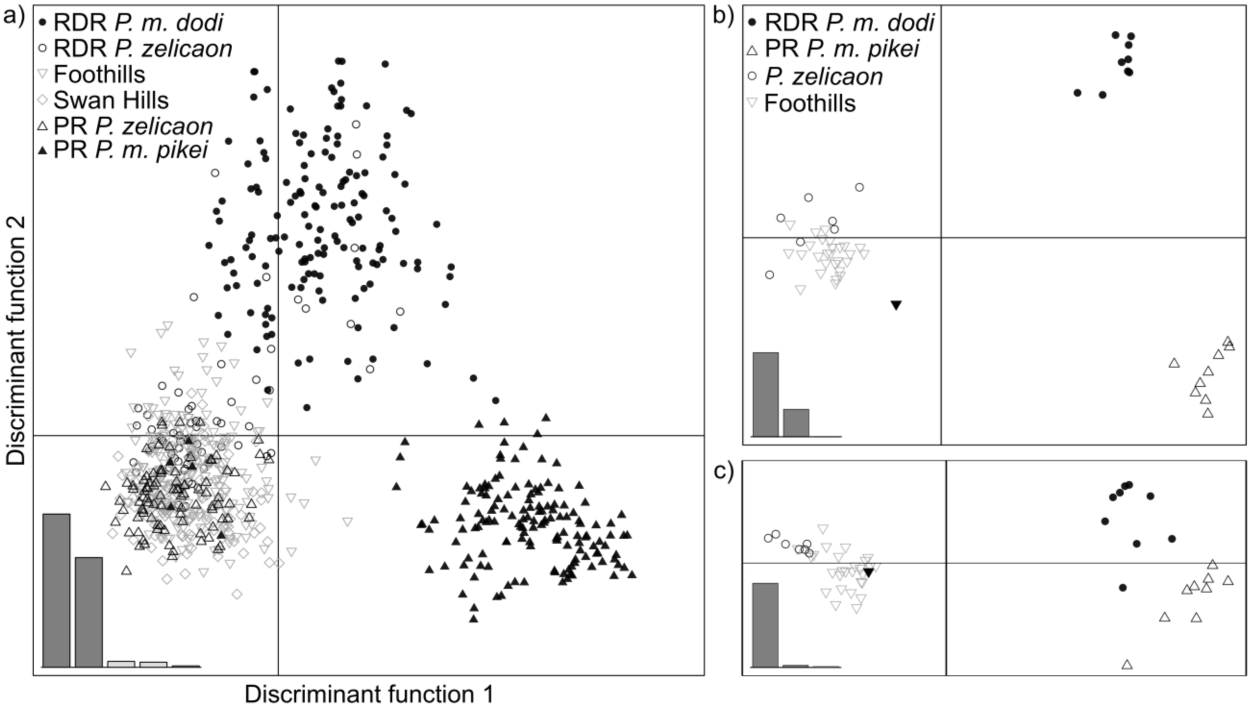

Figure 4.3 Discriminant analysis of principal components of a) microsatellites, b) all SNPs (1614 loci), and c) parentally informative SNPs (37 loci). Inset histogram in bottom left of each panel shows the relative contribution of discriminant functions, the first two of which are plotted here. The unique *P. m. pikei* hybrid is denoted with a filled in triangle in b and c. RDR: Red Deer River region; PR: Peace River region.

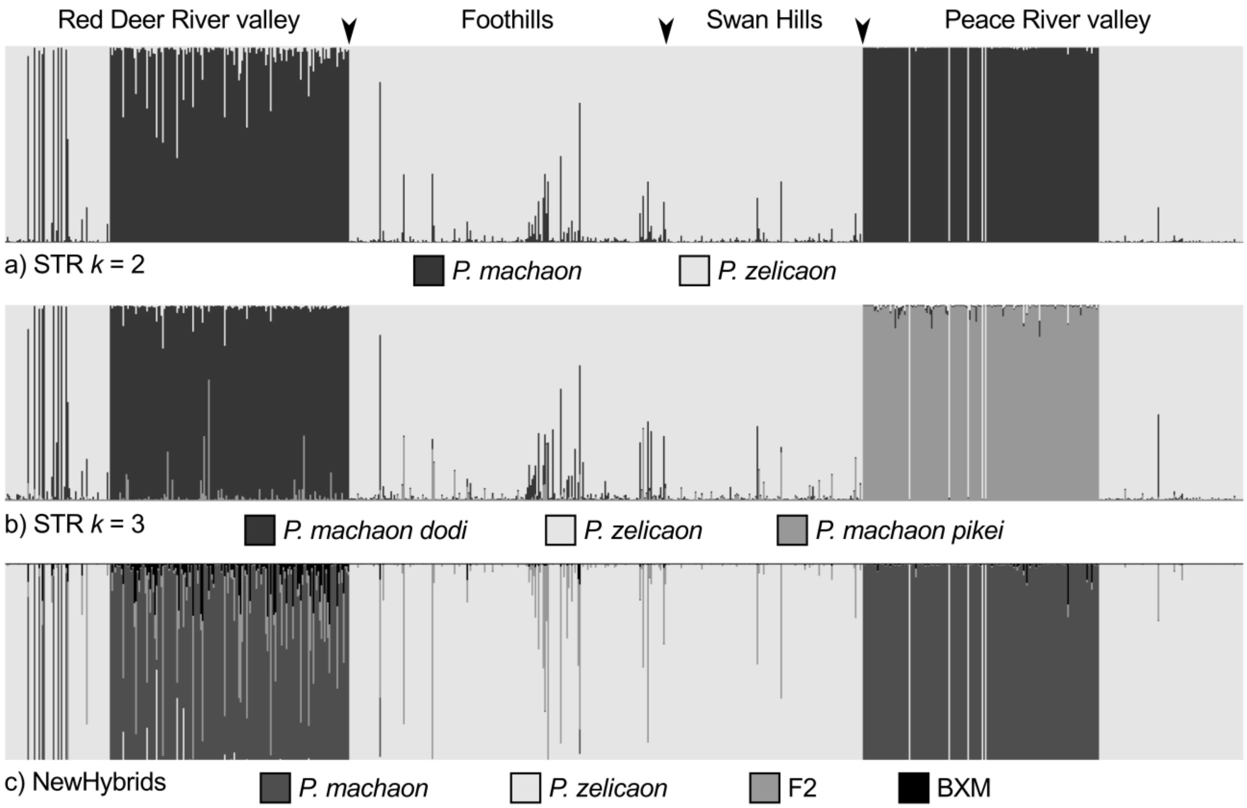

Figure 4.4 STRUCTURE ancestry (STR) and NewHybrids assignment estimates generated from microsatellite data: a) STRUCTURE $k = 2$; b) STRUCTURE $k = 3$; c) NewHybrids. Regions are demarcated by arrows above a.

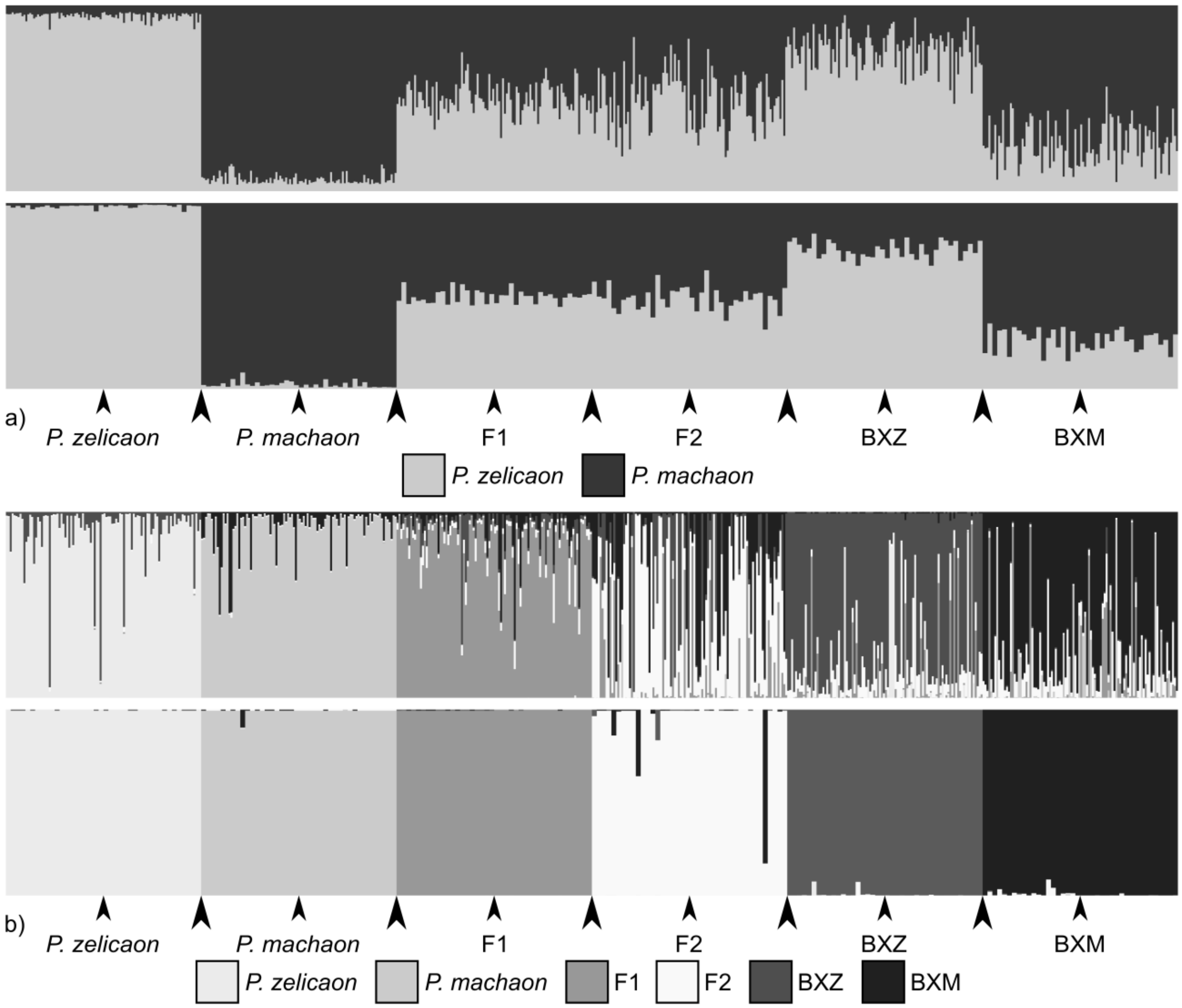

Figure 4.5 Example assignments of simulated datasets analyzed by: a) STRUCTURE $k = 2$, and b) NewHybrids, for microsatellites (upper panels in each pair) and parentally informative SNPs (lower panels). Large arrows below panels delimit simulated parental and hybrid classes, and small arrows indicate division between individuals simulated from the Red Deer River region (left of small arrow within each class) and Peace River region (right of small arrow within each class). Results for all replicates are in Appendices 4.5-4.8.

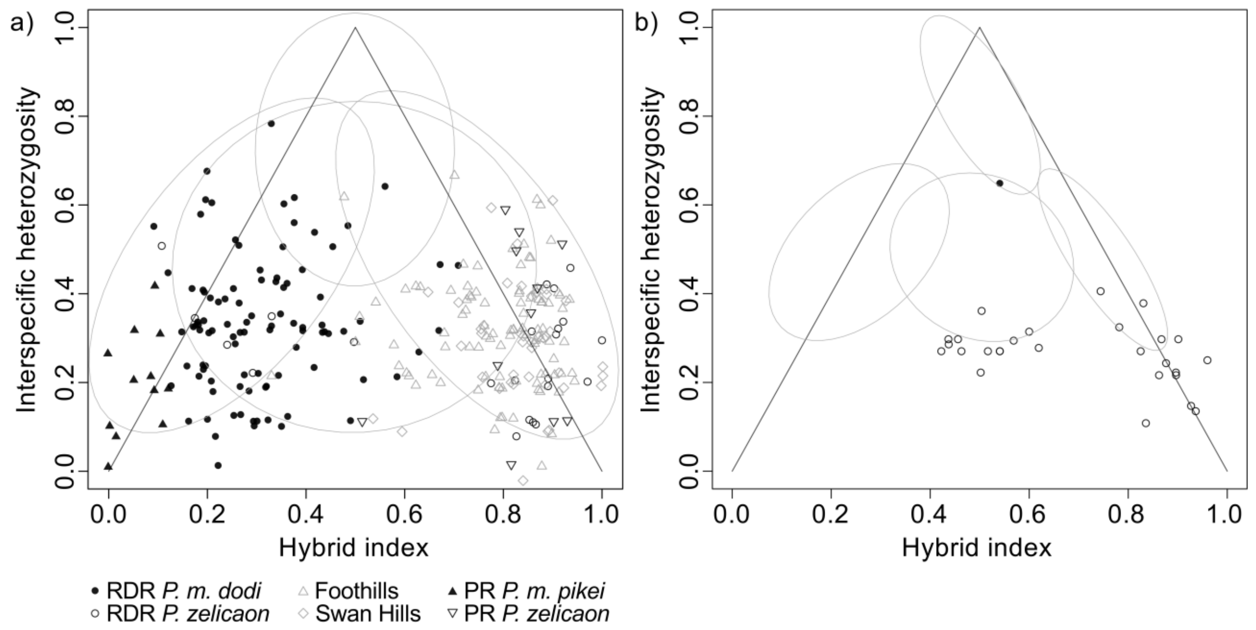

Figure 4.6 Joint distributions of ancestry (hybrid index, zero: *P. machaon*, one: *P. zelicaon*) and interspecific heterozygosity: a) microsatellite dataset of 280 admixed individuals, and b) parentally informative SNP dataset of 28 individuals from the Foothills region. Grey circles represent 95% confidence intervals for F1, F2, and backcross hybrid class created from the combined simulated datasets (2000 and 800 admixed individuals for the microsatellite and SNP datasets, respectively). Points in the microsatellite plot (left) have been jittered to aid in visualization of overlapping points, and symbols correspond to geographic regions. Points in the SNP plot (right) have not been jittered, and the unique *P. m. pikei* hybrid individual (see results) is noted as a filled circle; all other individuals are from the Foothills region. RDR: Red Deer River region; PR: Peace River region.

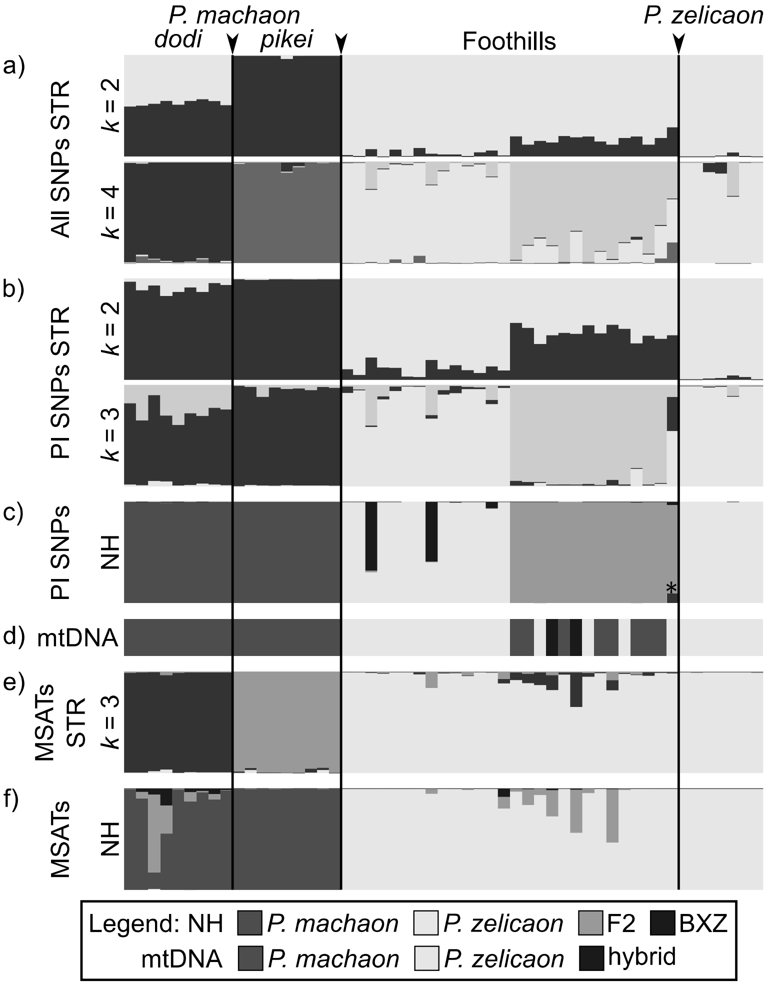

Figure 4.7 Assignments for the SNP dataset, showing SNP and associated COI haplotypes (mtDNA) and microsatellite (MSAT) assignments for the same individuals: a) STRUCTURE (STR) for all SNPs, b) STRUCTURE for parentally informative (PI) SNPs, c) NewHybrids for parentally informative SNPs, d) mtDNA clade, e) STRUCTURE for microsatellites, and f) NewHybrids for microsatellites. Shades distinguish genetic clusters for STRUCTURE results, hybrid classes for NewHybrids results, and mtDNA clades. The asterisk and dark bar in c) denotes the only F1 probability observed in the unique *P. m. pikei* hybrid (see methods), which is included with the Foothill region samples due to its hybrid nature. Back-cross to *P. zelicaon* (BXZ) probabilities in f) represent BXZ for Foothills region individuals, but back-cross to *P. machaon* (BXM) probabilities for *P. machaon* individuals.

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Chapter 5

Landscape effects on hybridization in a swallowtail butterfly species complex

5.1 Summary

Hybridization can be affected by myriad factors, from temporal and genomic characteristics to spatial influences, and sometimes disproportionately by landscape and environmental features. However, most research on hybrid zone formation and maintenance is restricted to analyses that relate hybrid occurrence to general landscape features or human disturbance; few studies on hybrid zones have attempted to combine research on hybridization with the newly emergent field of landscape genetics. Here we quantify the effect of landscape and environmental variables on hybridization by two swallowtail butterfly species in western Canada: *Papilio machaon* and *P. zelicaon*. We use redundancy analysis (RDA), raster-based resistance scheme optimization, and multiple regression on distance matrices (MRDM) with two genetic datasets (microsatellites and mitochondrial DNA), and consider spatial scales that address different levels of genetic differentiation in the system. Using RDA, cropland strongly explains genetic differentiation between parental species for both types of genetic markers, and acts as a surrogate variable for the larval habitat of *P. machaon*, which is arid river valleys that are often adjacent to cropland. At finer spatial scales, focusing on the main zone of hybridization, we observe more variable effects of landscape on genetic differentiation, although the presence of mixed forest explains higher admixture in microsatellites and unique hybrid mitochondrial haplotypes. Raster-based analysis identified highly variable results that depend on spatial scale and different measures of genetic distance and differentiation. Regardless of this variability, we observed high linear connectivity along the east slope of the Rocky Mountains, and more patchy

connectivity away from the Rocky Mountains. This is the first use of raster-based landscape genetic analyses (MRDM) in a hybrid system, and presents a conceptual shift from focus on landscape resistance surfaces affecting genetic structure within species to an integration of both landscape and genomic resistance to gene flow between species.

5.2 Introduction

Hybridization between divergent populations or species is increasingly recognized as both common and evolutionarily important (Butlin *et al.* 2012, Abbott *et al.* 2013). This process provides a novel source of adaptive variation both within and outside the context of speciation (Dasmahapatra *et al.* 2012, Hedrick 2013, Hamilton & Miller 2015) and can create stable or persistent hybrid zones when balanced with selection (Barton & Hewitt 1985, Nosil *et al.* 2009). Fueled by expanding genomic tools, modern research on hybridization has focused largely on the effects of endogenous factors in hybrid zones (e.g. hybrid viability, fecundity, genomic mosaicism, etc.), which has greatly accelerated our understanding of the dynamics of reproductive barriers in the face of gene flow (Smadja & Butlin 2011, Abbott *et al.* 2013), hybrid speciation (Rieseberg *et al.* 2003, Dasmahapatra *et al.* 2012) and variable genomic architecture (Teeter *et al.* 2009, Harrison & Larson 2014). Exogenous (environmental or landscape-based) factors have also played a key role in our understanding of hybridization, from early theories of hybrid zone maintenance and structure (Moore 1977, Barton & Hewitt 1985, Harrison & Rand 1989), to modern explanations for hybridization dynamics (e.g. Fitzpatrick & Shaffer 2007, Pfennig 2007, Hoban *et al.* 2012).

Despite the importance of exogenous factors in shaping hybrid zone dynamics, integration of analytical approaches from the growing field of landscape genetics (Manel *et al.*

2003) into hybridization research has progressed more slowly than the use of genomic tools. Landscape genetics combines landscape ecology, most often implemented using geographic information systems (GIS), with spatial statistics and population genetics to describe the relationship of evolutionary processes and landscape-scale spatial variables (Manel *et al.* 2003, Storfer *et al.* 2007, Manel & Holderegger 2013). By quantitatively incorporating landscape heterogeneity into assessments of gene flow and genetic differentiation, landscape genetics allows researchers to address functional connectivity, or how different landscape features facilitate or inhibit movement of individuals (Taylor *et al.* 1993, Baguette *et al.* 2013). This is most often accomplished through the use of raster-based analyses, where the landscape is divided into a grid of cells (via GIS) and each cell is assigned a value that represents the relative resistance of movement across that cell, according to a particular landscape characteristic (e.g. roads may inhibit black bear movement more than forested habitat, and therefore would have a higher resistance; Cushman *et al.* 2006). Conceptually, this incorporation of landscape heterogeneity extends the model of isolation-by-distance (genetic differentiation is positively correlated to Euclidean distance, IBD: Wright 1943) to the model of isolation-by-resistance (genetic differentiation is positively correlated to resistance distance, IBR: McRae 2006). Most often this is estimated using electrical circuit theory to model resistance distance according to landscape characteristics (McRae 2006, McRae & Beier 2007, McRae *et al.* 2008).

The IBR model has greatly increased our understanding of functional connectivity (Zeller *et al.* 2012, Manel *et al.* 2013), however research on hybridization has been slow to adopt methodological approaches to quantify IBR. Most studies of hybrid zones that have considered their landscape have done so by associating hybrid individuals to particular landscape features (Cruzan & Arnold 1993, Pfennig & Simovich 2002, Pfennig 2007, Larson *et al.* 2013), often in systems of invasive species and human disturbance (Fitzpatrick & Shaffer 2007, Walters *et al.* 2008, Hoban *et al.* 2012). While such associations can shed light on dynamics of hybrid systems, as well as selection pressures acting on hybrid individuals (e.g. Pfennig 2007), they do not address the comprehensive effect of an entire landscape on movement and gene flow. In this study, we assess the effect of landscape and environmental characteristics on gene flow in a system of hybridizing swallowtail butterflies in western Canada. We employ association-based modeling of populations with locality-specific environmental characteristics as well as raster-based analyses using IBR, which is the first application of the latter in a hybrid system.

The Old World swallowtail butterfly, *Papilio machaon*, and anise swallowtail, *P. zelicaon*, occur in sympatry and parapatry across western North America, and morphologically intermediate hybrid individuals have been collected from central and southwest Alberta, Canada, since the early 1900's (Scott 1986, Sperling 1987, Sperling 1990, Dupuis & Sperling 2015). *Papilio machaon* is represented in Alberta by two regionally common subspecies: *P. m. dodi* in the southern half of the province, and *P. m. pikei* in the Peace River valley of northwestern Alberta and adjacent British Columbia (Sperling 1987, Pelham 2008). Both subspecies are restricted to arid river valley habitats, where their larvae feed on *Artemesia dracunculus* on eroding riverbanks, and adults hilltop (mate-locating behavior where adults congregate on topographical features: Shields 1967) on prominent edges of the river valleys (Scott 1986). The habitat and larval host plants of *P. zelicaon* are more diverse in western Canada, but populations are centered on isolated hilltops in boreal forest, grasslands and foothills of the Rocky Mountains (Sperling 1987, Bird *et al.* 1995). Both species are highly vagile and despite their ecological differences and broad sympatry, *P. machaon* and *P. zelicaon* appear to hybridize freely in a few areas of Alberta. Several types of molecular markers have identified variable levels of genetic admixture in the foothills of southwest Alberta, suggesting both contemporary hybridization and historic hybrid influences from *P. m. hudsonianus*, a rarely encountered subspecies found in the boreal forest (Sperling 1987, Sperling 1990, Sperling & Harrison 1994, Dupuis & Sperling 2015, chapter 4).

Here we investigate the effect of landscape and environmental factors on gene flow and hybridization between *P. machaon* and *P. zelicaon* in Alberta and northeastern British Columbia. Using microsatellite and mitochondrial DNA (mtDNA) datasets (chapter 4), we use several methods of analysis to ask: 1) Can landscape or environmental variables explain their genetic diversity and differentiation? 2) Do landscape effects differ between nuclear and mitochondrial markers, spatial scales, genetic diversity, or genetic distance? Finally, 3) do landscape or environmental factors facilitate or inhibit hybridization between *P. machaon* and *P. zelicaon*? We consider three hierarchical spatial scales in our analyses, to allow us to also address genetic associations at smaller scales without the overarching influence of genetic divergence between species.

5.3 Methods

5.3.1 Collections, molecular methods, and spatial design

Sampling was conducted as in chapter 4, and microsatellite genotyping and DNA sequencing followed methods outlined by Dupuis & Sperling (2015). In brief, a total of 822 specimens were collected from four regions in Alberta and adjacent northwestern British Columbia: Red Deer River (southern Alberta grasslands), Peace River (northern Alberta and adjacent British Columbia grasslands), Swan Hills (north-central Alberta boreal forest), and the mainly forested Foothills of the Rocky Mountains in Alberta. Collecting in provincial parks in

Alberta and British Columbia was done under permit numbers 10-097 and 105180, respectively, and collecting on private land was done with the owner's permission. Genotype data was collected for 10 microsatellite loci originally developed by Zakharov & Hellman (2007), and an 831 basepair (bp) region of mitochondrial *cytochrome oxidase subunit I* (COI) was sequenced.

We consider three spatial scales for our analyses. The first includes all sampled populations, including both *P. machaon* and *P. zelicaon*. The second focuses on populations of *P. zelicaon* and hybrid populations, in order to simplify genetic structure for raster based analyses. The third includes only populations in the Foothills region of southwestern Alberta. We refer to these three spatial scales as "all populations", "*P. zelicaon* & hybrid", and "Foothills region", respectively. Using hierarchical scales allows us to address three levels of genetic differentiation; at smaller scales, such as in the Foothills region, fine-scale differentiation can be considered with less bias due to the greater genetic divergence between species.

5.3.2 Redundancy analysis

To test what geographic and environmental characteristics may drive gene flow between populations, we conducted redundancy analysis (Rao 1964). This method is a multivariate extension of multiple linear regression (Legendre & Legendre 2012), and explores variation in a set of predictor variables that explains the variation present in a set of response variables. In our case, population-based genetic data was the response variable and environmental characteristics at each locality were predictors. For the microsatellite dataset, allele counts in each population were transformed to Hellinger distances (Legendre & Gallagher 2001) using the `decostand` function in the vegan package v2.2-0 (Okansen *et al.* 2014) in R v3.1.1 (R Core Team 2014). This transformation was developed for species abundance data, and is appropriate for microsatellite data with high allelic diversity, and thus many missing values (absent alleles) in individual populations (Cullingham *et al.* 2014). For COI, we conducted distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999), using a matrix of pairwise Jost's D statistics (Jost 2008) calculated from FASTA formatted sequences with the R packages APE (Paradis *et al.* 2004) and mmod (Winter 2012). An alignment of 594 bp was used, which eliminated variable missing data from the 3' and 5' ends of the sequencing product.

Environmental and landscape characteristics of each population locality were used as predictors in the RDA/dbRDA; data sources, rationale and general predictions for each variable type, are given in Table 5.1. When possible, we chose several measures of a general variable type (e.g. temperature, precipitation), to provide optimal explanatory power for subsequent analyses. To avoid problems associated with multicollinearity (Graham 2003), we filtered the full set of environmental variables by first removing variables correlated above 0.90. We then removed additional variables that met two criteria: 1) those that explained a low amount of the variance in the genetic data (less than 5%), assessed with variance partitioning (see below, Borcard *et al.*1992, ter Braak & Verdonschot 1995) conducted with the vegan package in R; and 2) those with a variance inflation factor (VIF: Zuur *et al.* 2009) >10, calculated with the car package (Fox & Weisberg 2011) in R. This approach resulted in sets of environmental variables with low multicollinearity (VIF<10) and high explanatory power for the genetic data, and retained more environmental variables than would have been retained with a lower initial correlation threshold. We also wanted to distinguish between the individual variability explained by each environmental variable, and any shared contribution of multiple variables explaining genetic variation. To do so we conducted variance partitioning on three categories of variables: geography (latitude, longitude, and elevation), climate (generated with ClimateNA, Hamann *et* *al.* 2013), and habitat (broad-scale land cover). This method separates the total variation explained in the genetic data into variation explained by individual variable categories and combinations of variable categories. To assess significance of the variable categories, we conducted additional RDA/dbRDA on categories individually and used analysis of variance (ANOVA) in R with 999 permutations. Redundancy analysis and dbRDA, as well as variance partitioning, were conducted at two spatial scales: all populations and the Foothills region.

5.3.3 Landscape resistance and effective distance measures

To incorporate aspects of landscape between population localities, we implemented GIS-based model optimization by creating a set of resistance surfaces for environmental characteristics of interest. By testing alternative models of landscape resistance for each environmental variable, and correlating these models to various measures of gene flow, we can identify which landscape features facilitate or inhibit gene flow, as well as infer the relative importance of these characteristics to movement across the landscape (e.g. Pérez-Espona *et al.* 2008). This method is appropriate for our study system, where there is little *a priori* information regarding the cost of dispersal for individuals in the landscape (Cushman *et al*. 2006, Pérez-Espona *et al*. 2008, Spear *et al*. 2010). Since many of the climatic variables used for the RDA are thematically related (e.g. temperature, precipitation, etc.: Table 5.1) and displayed high correlation among thematic variables, we chose a subset of these to cover all major variable types: fine-scale land cover, mean annual temperature (MAT), mean annual precipitation (MAP), extreme minimum temperature over 30 years (EMT), and slope. Fine-scale land cover was inferred from a GIS resource created for forestry (Table 5.1), and was unavailable for the southeastern portion of this study area (grassland regions around the Red Deer River). Therefore, for this analysis we omitted populations from the Red Deer River region, as well as *P. machaon pikei* populations from the Peace River region. Removal of all predominately pure *P. machaon* populations simplifies the genetic and geographic complexity in the system for raster-based analysis (namely, occurrence of two regions of *P. machaon* at opposing ends of the study area, separated by a swath of *P. zelicaon*); this simplification allowed us to focus on subtler genetic differentiation associated with hybrids of predominately *P. zelicaon* ancestry, rather than the large differentiation between *P. machaon* and *P. zelicaon*. We refer to the spatial scale excluding the Red Deer River populations and those of *P. m. pikei*, as the "*P. zelicaon* and hybrid" scale. Slope, rather than elevation, was used for this analysis as it more accurately reflected *a priori* hypotheses relating to the strong hilltopping behavior of these butterflies (Sperling 1987, Sperling 1990), and was calculated from the same digital elevation model as in Table 5.1. All raster layers were standardized to 500 m spatial resolution using ArcGIS (ESRI 2011).

Most studies that have used this type of analysis to investigate which landscape features affect gene flow have done so with discrete features (e.g. roads, rivers, forest vs. non-forest, etc.) and ground-travelling species in small geographic areas (Cushman *et al.* 2006, Pérez-Espona *et al*. 2008, Pérez-Espona *et al*. 2012). In these cases, researchers can objectively build a set of resistance surfaces in which the resistance of the feature of interest is varied against a background that is held constant (e.g. the resistance of road cells is set anywhere from 0.1 to 100, and all background cells are set to 1). Here, we assessed gene flow across a large geographic area, with mainly continuous environmental variables (e.g. aspects of climate and slope), and highly vagile, flying organisms, which we did not expect to be affected by small linear features. Therefore, we adjusted our approach to building resistance surfaces.

For each environmental variable, we created five resistance schemes to test the effect of low resistance across the range of values observed in our study area. In these resistance schemes, the observed range of values across the study area were divided into 10 equal categories in ArcGIS, and in each scheme a different low-resistance set of values were chosen and assigned a resistance of 1. As values increased or decreased away from this range of low resistance, we assigned an increasing value of resistance to a maximum resistance of 100. This resulted in five resistance schemes that represent a linear progression through the range of variable values. We refer to these as the “linear resistance schemes” and number them as resistance schemes one through five. We then created five additional schemes to test *a priori* hypotheses about swallowtail butterfly movement (following predictions in Table 5.1), by varying the extent of low-resistance cells within the range of variable values. These schemes were numbered six through ten. Fine-scale land cover is a discontinuous variable when represented in a raster, and we therefore only tested five resistance schemes focusing on hypotheses of butterfly movement (in this case numbered schemes one to five). Resistance schemes are summarized in Supplementary Table 5.1. Pairwise modeling in CIRCUITSCAPE v 4.0.5 (McRae 2006, McRae & Beier 2007, Shah & McRae 2008) was then used to calculate the effective distance between populations given each of the resistance schemes. Unlike least cost path analysis (Andriaensen *et al.* 2003) that calculates an effective distance based on a single optimal path, CIRCUITSCAPE uses circuit theory to model resistance across all possible paths across a landscape (McRae 2006), and is more representative of an IBR model of connectivity. Resistance schemes that were not variable for the populations in question (null resistance schemes) were omitted.

5.3.4 Resistance scheme optimization and genetic distance measures

The effective distances generated from each landscape resistance scheme were correlated to measures of genetic distance (see below) using Mantel tests (Mantel 1967), implemented in the vegan package of R with 10,000 permutations to assess statistical significance. The resistance scheme with the highest correlation from each environmental variable was used to assess the effect of that variable on gene flow (whether it facilitated or inhibited gene flow, or had no affect). While the Mantel test has been shown to have high type I error (Balkenhol *et al.* 2009), it is the most commonly used statistical test in landscape genetics and conservative application of Mantel tests for distance-based data has been shown to be appropriate (Cushman *et al.* 2006, Pérez-Espona *et al*. 2008, Legendre & Fortin 2010, Pérez-Espona *et al*. 2012).

Several measures of genetic distance were compared in the resistance scheme optimization. For the microsatellite dataset, Nei's D (Nei 1978) and G''_{ST} (Meirmans & Hedrick 2011) were calculated in GENODIVE (Meirmans & Van Tienderen 2004). F_{ST} (Meirmans 2006) and Jost's D (Jost 2008) were also calculated in GENODIVE, but results did not differ substantially from G''_{ST} and Nei's D for this dataset, so these statistics were not pursued. Nei's D and G''_{ST} were chosen as they are both standardized values (taking into account both small sample size, and for G''_{ST}, sampling of a limited number of populations: Meirmans & Hedrick 2011), and to provide alternative assessments of population differentiation (Verity & Nichols 2014). We also calculated a genetic distance for microsatellites based on the average ancestry of each population, determined from STRUCTURE (Pritchard *et al.* 2000). For this distance, the *Q* values of the *P. zelicaon* cluster from the $k = 2$ results (chapter 4) were averaged for every population, and a pairwise absolute distance (Manhattan distance) matrix was created with the `dist` function and the MASS (Venables & Ripley 2002) package in R. Integrating quantitative measures of individual ancestry or population subdivision in landscape genetic analyses is a recently developed approach, and has been successful at elucidating the effects of landscape at fine scales and in hierarchically organized systems (Balkenhol *et al.* 2014, Anderson *et al.* 2015, Cullingham *et al.* 2015). Here, we use this approach as a quantitative measure of hybrid prevalence in populations, as a lower average *Q* value for a population (pure *P. zelicaon Q* would equal one, and pure *P. machaon* zero) indicates a higher prevalence of mixed ancestry. We refer to this measure as "*Q*-distance". For COI, we used the same matrix of Jost's D values as was used for the dbRDA. Resistance scheme optimization was conducted at the scale of *P. zelicaon* and hybrids, as well as the Foothills region.

5.3.5 Multiple regression on distance matrices

We conducted multiple regression on distance matrices (MRDM: Legendre *et al.* 1994, Legendre & Legendre 2012) of genetic and effective distance to develop optimal landscape genetic models for the different genetic distance measures and spatial scales. This approach tests the individual and combined effects of multiple landscape variables (via effective distance matrices) on genetic distances, and has been shown to be a reliable method (high power and low error) in landscape genetics studies (Balkenhol *et al.* 2009, Balkenhol *et al.* 2014). MRDM was conducted with the ecodist package (Goslee & Urban 2007) in R using 10,000 permutations to assess statistical significance. All genetic distance measures were analyzed separately for the *P. zelicaon* and hybrid and Foothills region scales, and we used the resistance scheme for each environmental variable that had the highest correlation to that particular genetic distance in the resistance scheme optimization. We used a modified forward, stepwise selection procedure with a *P*-to-enter value of 0.1 (Balkenhol *et al.* 2009) to remove variables that did not contribute significantly to the model. We modified the forward, stepwise selection procedure to differentiate between variables that did not contribute to the model and the effects of multicollinearity. When collinearity was detected between variables (VIF > 10 or a significant correlation in a Mantel test between the variables), partial Mantel tests (conducted using ecodist with 10,000 permutations) were used to identify which variable better explained the genetic diversity, and that variable was included in the model. We compared the results of the forward, stepwise selection procedure with those of backward elimination of variables, as we observed several cases of individual variables that had insignificant individual effect in the model, but significant effect when paired with other uncorrelated variables. For the final models from MRDM, we created current maps using CIRCUITSCAPE to visualize the resulting model. In cases of models with multiple environmental variables, we summed and averaged the resistance values for each cell to create a cumulative resistance surface, but this did not have an effect on the final current map, so we present the results of the summed model. Multiple regression on distance matrices was conducted at two spatial scales: *P. zelicaon* and hybrids as well as the Foothills region scale.

5.4 Results

5.4.1 Redundancy analysis

The final set of variables included as predictors in the RDA/dbRDA of all sampled populations were latitude, elevation, broad-scale land cover, mean annual precipitation (MAP), mean coldest month temperature (MCMT), and extreme minimum temperature over 30 years (EMT). All three categories of variables (geographic, climatic, and habitat-based) independently explained a significant proportion of the microsatellite allelic variation (adjusted $R^2 = 0.1517$, 0.1307, and 0.2066, respectively; all $P < 0.001$), and COI distance variation (adjusted $R^2 =$ 0.1578, 0.2251, and 0.1437, respectively; $P < 0.001$, $P < 0.001$, and $P = 0.009$, respectively). However, variance partitioning identified a large proportion of genetic variation that was explained by shared variation between the three variable classes for both microsatellites (adjusted $R^2 = 0.1037$; Figure 5.1a) and COI (adjusted $R^2 = 0.09959$; Figure 5.2a). Climatic variables explained virtually no variation in microsatellite allelic diversity that was not also explained by geography or habitat (Figure 5.1a), but this pattern was not observed with COI (Figure 5.2a). Visually, microsatellite-based genetic variation between *P. machaon* and *P. zelicaon* were best explained by broad-scale land cover (specifically cropland), and the separation of *P. m. dodi* and *P. m. pikei* by latitude (Figure 5.1b). Cropland also explained the genetic separation of *P. machaon* and *P. zelicaon* with COI (Figure 5.2b). Combinations of land cover characteristics and climatic variables visually explained some of the divisions between regions of *P. zelicaon* ancestry for both microsatellites and COI, although less genetic separation was observed so the effect of environmental variables was more difficult to determine.

When only populations in the Foothills region were considered, the final set of predictors included latitude, broad-scale land cover, mean annual temperature (MAT), and mean summer precipitation (MSP). Geography (latitude) explained a significant proportion of variation in the microsatellite and COI datasets (adjusted $R^2 = 0.0307$ and 0.2452, $P = 0.01$ and 0.012, respectively), while climatic and habitat-based variables did not (climatic variables: adjusted R^2 $= 0.02489$ and 0.01163, $P = 0.123$ and 0.378, for microsatellites and COI, respectively; habitat-based variables: adjusted $R^2 = 0.00741$ and 0.00305, $P = 0.385$ and 0.453, for microsatellites and COI, respectively). No variation was shared between pairs of these categories for microsatellites, but a substantial amount of variation explained by the predictors was shared between all variable categories (Figure 5.1a). Conversely, for COI there was no explained variation shared by all three variable categories, and little variation explained by the categories individually (Figure 5.2a). Visually, MSP and mixed-forest land cover (LCmix) seemed to best explain the presence of admixed individuals with microsatellite data (Figure 5.1c), and LCmix best explained the presence of *machaon*-like and hybrid COI clades based on genetic distance (Figure 5.2c).

5.4.2 Resistance scheme optimization

Five resistance surfaces were null (non-variable for the population localities in question) for the *P. zelicaon* and hybrid-scale dataset and seven for the Foothills region-scale dataset. The four genetic distance measures, Nei's D, G''_{ST}, and *Q*-distance (for microsatellite data), and Jost's D (for COI data), resulted in 16, seven, four, and five significant Mantel tests for the *P. zelicaon* and hybrid-scale dataset, respectively, and 12, 15, zero, and 17 for the Foothills region-scale dataset, respectively (Appendix 5.1). For the linear resistance schemes, correlations of the four genetic measures were largely congruent (Figure 5.3); for the *P. zelicaon* and hybrid scale, Jost's D (COI) showed the most differences, while for the Foothills region scale, the correlation patterns for *Q*-distance did not conform to the other genetic measures. Selecting the resistance scheme for each variable that had the highest significant R^2 value resulted in between one and four variables for the *P. zelicaon* and hybrid-scale dataset and between zero and five for the Foothills region-scale dataset (Table 5.2). Despite the congruent patterns observed in the correlations of the five linear resistance schemes, there was variability in the most highly correlated scheme for each genetic measure (Table 5.2).

5.4.3 Multiple regression on distance matrices

When considering the resistance schemes with the highest R^2 value (ignoring their significance) for each genetic measure, we identified high correlation between environmental variables, and thus fewer variables were included in the final models using MRDM (Table 5.3). All genetic measures for the *P. zelicaon* and hybrid-scale dataset resulted in models with two environmental variables, and for populations in the Foothills region, models for three of the genetic measures included a single variable, while one genetic measure did not result in any models being supported (Table 5.3). In one of the latter models, a single variable had a VIF >10, but significant results in the MRDM and Mantel test validation, so we included it in the final model. Resistance schemes for mean annual temperature (MAT) were the most frequently observed compared to other environmental variables in the final MRDM models, although there was substantial variability in the environmental variables that were included in the final models for each genetic measure (Table 5.3).

5.4.4 Comparing raster-based analyses

For the raster-based analyses, there was substantial variability and incongruence in which environmental variables best explain genetic diversity. While several environmental variables produced consistent results across analysis type (resistance scheme optimization and MRDM) and genetic measure (including both microsatellites and COI), others did not (Table 5.2, Table 5.3). The results for slope were the most consistent of the environmental variables, and all supported resistance schemes had lower resistance values assigned to increased slope values. Mean annual precipitation (MAP) was relatively consistent, with moderate to high levels of precipitation corresponding to lower resistance.

Land cover, mean annual temperature (MAT), and extreme minimum temperature over 30 years (EMT) displayed inconsistent results when the significant resistance schemes of the optimization process were compared to those from the MRDM. Land cover and EMT were particularly inconsistent, with contrasting resistance schemes being positively or negatively correlated with different genetic distance measures (Table 5.2, Table 5.3). Mean annual temperature had less inconsistent correlations (all were positive relationships), but the supported resistance schemes, at least from the optimization procedure, ranged from schemes where high temperature had low resistance to ones where high temperature had high resistance. There was less inconsistency when only MRDM results were considered, as well as support for schemes where moderate to high temperatures correspond to low resistance.

The final supported models from the MRDM were used to create resistance surfaces (current maps) in CIRCUITSCAPE (Figure 5.4). For many of the final MRDM models, there was an obvious strong effect of the Rocky Mountains on our assessments of connectivity, which is unsurprising given the environmental variables included in those models: Mean annual and extreme minimum temperature (MAT, EMT), slope, and mean annual precipitation (MAP) would all be highly correlated with mountainous habitats. In these models, there was high north-south connectivity along the Foothills of the Rocky Mountains, and east-west connectivity from localities south of the Peace River valley to those in the Swan Hills. A similar path of connectivity along the east slope of the Rocky Mountains was observed at the Foothills region scale, with additional connectivity between populations in the northeast corner of this extent. Interestingly, the only final MRDM model without obvious effects of the Rocky Mountains was that of Jost’s D (COI) for the larger spatial scale (*P. zelicaon* and hybrids). In this case, open land cover classes (grassland, agriculture, etc.) appeared to facilitate gene flow in a fashion more typically observed in landscape genetics studies, but this connectivity extended from the southern localities with north-northeast directionality, through the Swan Hills and into localities south of the Peace River valley.

5.5 Discussion

Here we use spatial ecology and landscape genetics approaches to quantify the affect of landscape and environmental variables on gene flow between species, as measured by the presence of hybrid individuals, in the *P. machaon* species group in western Canada. While we identify environmental variables that explain the genetic diversity in this system, we also find that the choice of genetic distance measure and spatial scale greatly influence results, particularly for raster-based analyses.

We identified a combination of environmental and landscape variables that explain genetic differentiation between *P. machaon* and *P. zelicaon*. The presence of cropland explained this differentiation most clearly with both genetic datasets using RDA/dbRDA (Figure 5.1b, Figure 5.2b), which may seem counterintuitive at first. Both of these butterflies may be found nectaring as adults in agricultural settings (Dupuis *personal observation*), but neither species specializes on agricultural habitats (Scott 1986, Sperling 1987), and many cropland habitats are considered unsuitable for insect habitation and dispersal (e.g. Ouin *et al.* 2004). However, the adults of *P. machaon* populations commonly occur on the tops of the arid river valleys of the Red Deer and Peace Rivers, directly adjacent to, and in some cases within steps of, agricultural cropland. In these analyses the proximity of cropland acts as a surrogate variable for the arid river valley habitat used by *P. machaon* and its host *Artemesia dracunculus* (Sperling 1987). Host occurrence has been shown to have primary roles in butterfly movement and population dynamics (Hanski & Heino 2003, Fred *et al.* 2006). We expect that a variable indicating host-plant occurrence would also be highly explanatory for these species distributions. Unfortunately, accurate, high-resolution host plant data on a multi-province scale was unavailable for this study.

At a finer scale, we observed less distinction between regions of predominately *P. zelicaon* ancestry using RDA/dbRDA (Figure 5.1b, Figure 5.2b). Microsatellite allelic variation is partially explained by combinations of landscape and environmental variables (e.g. deciduous forest (LCdec) and mean annual precipitation (MAP) for the Swan Hills region, and rangeland (LCrange) and variables of minimum temperature (EMT, MCMT) for *P. zelicaon* in the Red Deer River valley; Figure 5.1b), which are reasonable given the characteristics of these geographic regions. In contrast, the high level of retained ancestral polymorphism in the COI dataset (individuals from across the ranges of *P. machaon* and *P. zelicaon* share identical haplotypes: Dupuis & Sperling 2015) precludes further meaningful interpretation at this spatial scale. In the Foothills region the mixed forest land cover class (LCmix) explains both higher admixture in microsatellites using RDA, and the presence of hybrid-like and *P. machaon* COI haplotypes using dbRDA (Figure 5.1c, Figure 5.2c). This variable may relate to historic or occasional introgression from the rarely collected *P. m. hudsonianus*, which is more commonly encountered in partially closed forest habitats (Sperling 1987, Dupuis & Sperling 2015). Fine scale land cover was also supported in the final MRDM model for Jost's D (COI) at the scale of *P. zelicaon* and hybrid populations (Table 5.3, Figure 5.4). We expected closed, forested habitats to impede movement and gene flow in this system given the behavior of these swallowtail butterflies (Dupuis & Sperling 2015) and previous findings in related species (Roland *et al.* 2000, Keyghobadi *et al.* 2005), but surprisingly this variable was not supported by MRDM of microsatellites using any genetic measure.

Although we observed high variation in the variables included in the final models of the resistance scheme optimization and MRDM (discussed below), we can assess our *a priori* hypotheses about how environmental variables affect gene flow on an individual basis. Our hypotheses for slope were largely supported: resistance schemes where higher slopes had lower resistance were optimal in our resistance scheme optimization. Given the strong hilltopping behavior of these butterflies (Scott 1986, Sperling 1987, Dupuis & Sperling 2015), this result is not surprising. Our hypotheses that increased precipitation will increase connectivity and gene flow were partially supported; while the optimal resistance schemes had lower resistance for moderate to high levels of precipitation, resistance schemes in which the maximum amount of precipitation had the lowest resistance were not supported (Appendix 5.1). Drought can severely impact butterfly populations, largely through its effects on host condition and survival (Ehrlich *et al.* 1972, Wallner 1987, Morecroft *et al.* 2002, Raimondo *et al.* 2004, Robinson *et al.* 2012), and more complex interactions between temporal variation of precipitation and butterfly population fluctuations (e.g. the current year's precipitation combined with the previous year's) have also been shown (Roy *et al.* 2001). Although our climatic data are modeled averages (Hamann *et al.* 2013), incorporation of direct temporal variation in these variables may increase the resolution of our results.

Unfortunately, the high variability of the supported resistance schemes (with both the optimization method and MRDM) precludes unequivocal conclusions on the effects of temperature (mean annual and extreme minimum temperature) on butterfly movement in this system. In the resistance scheme optimization, some genetic measures indicate that high temperature appears to facilitate movement (agreeing with our hypotheses), while we found the opposite with other measures (Table 5.2). When only the results of the MRDM were considered, moderate to high mean annual temperature and a *lower* extreme minimum temperature had lower resistance (Table 5.3). Interestingly, Sperling (1987) concluded that mean annual temperature (based on records from 1951-1980) better explained the distributions of many taxonomic entities in the *P. machaon* species group (particularly subspecies of *P. machaon* in Alberta & British Columbia), and that mean annual precipitation was more homogenous for the species and subspecies in this region. This apparent contradiction between Sperling (1987) and the present study could indicate temporal changes in these variables (the climatic data here is primarily derived from the period from 1961-1990), or differences due to methodology. However, we feel that the narrower scope of analysis in the current study likely plays a larger role, particularly with regard to the raster-based analyses used here, which omitted *P. machaon* and thus the range of systematic diversity considered by Sperling (1987). Regardless, both warmer and cooler temperatures have been shown to facilitate butterfly population dynamics depending on the species in question (and the sensitivity of the host plant to high temperatures and drought: Roy *et al.* 2001), and like precipitation, incorporating temporal variation may elucidate more fine-scale patterns in this system.

The impact of the Rocky Mountains on these raster-based analyses, which is particularly evident in the final MRDM resistance surfaces (Figure 5.4), is an important consideration when drawing conclusions about butterfly movement in this system. Given the simplicity of the final models (all including no more than two variables), the connectivity displayed in these resistance surfaces may simply represent artifacts of the Rocky Mountains on these few climatic variables, rather than true connectivity in the system. Alternatively, the ability to detect influences of landscape features can depend on the uniformity of habitat connectivity and the contrast between highly resistant and optimal landscape (Short Bull *et al.* 2011, Cushman *et al.* 2012), as well as the distribution of sampled populations (Storfer *et al.* 2007, Schwartz & McKelvey 2009). Given the uniform nature of many of the climatic variables used here when the Rocky Mountains are ignored, we could be biasing our results by inappropriately selecting environmental and landscape variables. For future research, it may be necessary to investigate more complex ecological models that could parse the effects of the Rocky Mountains from some of the environmental and landscape variables included in the analysis, although such an approach is beyond the scope of this study.

5.5.1 The effect of spatial scale and genetic distance measure

We found a large effect of different spatial scales and genetic measures on our interpretation of how landscape and environmental variables affect genetic differentiation and gene flow in this system; both aspects of this phenomenon have been addressed in the literature (e.g. Mullen *et al.* 2010, Angelone *et al.* 2011, Galpern *et al.* 2012, Keller & Holderegger 2013, Keller *et al.* 2013). Given the importance of spatial scale on landscape genetic analysis (Anderson *et al.* 2010, Cushman & Landguth 2010), it has been suggested that analyses should be limited to spatial scales where direct gene flow occurs (Murphy *et al.* 2010, Angelone *et al.* 2011, Keller *et al.* 2013) or to population groupings including only neighboring populations (Jaquiéry *et al.* 2011). With complete sampling of populations and better known organismal biology, it is possible to evaluate the best spatial scale for analyses (Keller *et al.* 2013). Unfortunately, in this system, characteristics such as deme size and maximum dispersal capability, as well as the completeness of our sampling, are unknown, so there is not necessarily a clear method to identify which spatial scale is most accurate. While we cannot eliminate the potential for misleading results due to either low power of analyses to detect landscape genetic patterns (Cushman & Landguth 2010) or bias due to incomplete sampling (Schwartz & McKelvey 2009), these results give us a starting point to consider the effect of landscape and environmental features on movement and gene flow in this system. Future research efforts should focus on more even sampling of populations at moderate spatial scales to aid in the interpretation of these results.

Many landscape genetic studies use multiple measures of genetic distance in their analyses (Storfer *et al.* 2010 and references therein), and often find variable results or differences in model performance using different measures (e.g. Pérez-Espona *et al.* 2012, Keller *et al.* 2013, Zancolli *et al.* 2014). These differences are expected, given the varied assumptions of the different measures (Jost 2008, Meirmans & Hedrick 2011, Verity & Nichols 2014), and can provide insight into both population and landscape genetic inferences. In our resistance scheme optimization and MRDM, we observed high variability across genetic measures for each spatial scale. Interestingly, despite this variability, many of the final MRDM models created similar cumulative resistance surfaces: High linear connectivity was associated with the east slope of the Rocky Mountains, and more patchy connectivity was observed in the area between localities south of the Peace River valley and those in the Swan Hills, as well as the northeast portion of the Foothills region. Both of these latter areas are characterized by variable boreal forest, rangeland, and agriculture (not ideal or continuous habitat for these butterflies), which may explain the moderate connectivity here compared to the east slope of the Rocky Mountains. Given the high level of correlation between the landscape and environmental variables used for these analyses, the apparent discrepancy in resistance schemes in these final models may simply be an artifact of slight differences in the correlations between variables leading to different final variables in the MRDM models (e.g. MAT and MAP may be highly correlated, but each may be slightly more correlated to individual genetic measures, leading to alternate variable selection in the final model). This scenario would lead to high similarity in final resistance surfaces despite different variables being present in the final models.

The obvious discrepancy from this scenario is the final MRDM model for Jost's D (COI) for the *P. zelicaon* and hybrid population scale, which includes fine-scale land cover as an explanatory variable and creates a very different resistance surface (Figure 5.4). We expected to see substantial differences when comparing measures of microsatellite genetic distance from those of mtDNA, based on the different evolutionary characteristics of these markers (e.g. Hedrick 1999, Ballard & Whitlock 2004), as well as the high degree of retained ancestral polymorphism in the mtDNA dataset (Dupuis & Sperling 2015). However, this broad difference may speak for the differential evolutionary histories of mtDNA versus the nuclear genome (Ballard & Whitlock 2004), although the same pattern was not apparent at smaller spatial scales. Interestingly, we also observed more congruence between markers with RDA and dbRDA, so this marker-specific discrepancy also depends on the analysis in question.

This research is the first use of raster-based landscape genetics methods in a hybrid system. Methodologically this is accomplished with hierarchical spatial scales to address different levels of genetic differentiation. Measures of gene flow or genetic distance still serve as the fundamental biological parameter that is related to landscape variation. This approach requires a conceptual shift, however, as gene flow between species can behave very differently than gene flow within a species. Such integration of landscape genetics methods into multi-species or community-level interactions (e.g. James *et al.* 2011, Hand *et al.* 2015) represents a new direction for the integration of landscape genetics and evolutionary biology.

Table 5.1 Environmental and landscape characteristics used in this study, their rationale, and broad predictions for major variable types. Acronyms are defined in the Description column.

Variable	Description	Source	Rationale for variable choice & predictions
Latitude	Northing in decimal degrees	GPS-determined	Latitude and Longitude incorporate sampling localities
Longitude	Easting in decimal degrees	GPS-determined	
Elevation	(in meters)	NOAA NCEI GLOBE DEM: http://www.ngdc.noaa.gov/mgg/topo/globeget.html	Topological prominences are used as congregation and mating locations in hilltopping butterflies, so we expect these butterflies to be drawn to higher elevation.
MAT	Mean Annual Temperature (°C)	ClimateNA v5.21 (Hamann *et al.* 2013)	As ectotherms, temperature plays a major role in insect development, distribution, and behavior (Bale 1991). Extreme cold temperatures affect overwintering survival (e.g. Marshall & Sinclair 2015) and warm temperatures and degree-day accumulation have various effects on growth and development (e.g. Wilson & Barnett 1983, Wallner 1987, Gilbert & Raworth 1996). We expect warm temperatures to facilitate population growth and butterfly dispersal, and extreme cold temperatures to do the opposite.
MWMT	Mean Warmest Month Temperature (°C)	ClimateNA v5.21	
MCMT	Mean Coldest Month Temperature (°C)	ClimateNA v5.21	
DD<0	Degree-Days below 0°C	ClimateNA v5.21	
DD>5	Degree-Days above 5°C	ClimateNA v5.21	
DD<18	Degree-Days below 18°C	ClimateNA v5.21	
EMT	Extreme Minimum Temperature (°C) over 30 years	ClimateNA v5.21	
FFP	Frost-Free Period	ClimateNA v5.21	
MAP	Mean Annual Precipitation (mm)	ClimateNA v5.21	Precipitation has wide-ranging effects on herbivorous insect population dynamics, largely through drought effects on host plants (Ehrlich *et al.* 1972, Wallner 1987, Morecroft *et al.* 2002, Raimondo *et al.* 2004, Robinson *et al.* 2012). We expect swallowtail butterfly dispersal to be facilitated by increased precipitation.
MSP	Mean Summer (May to September) Precipitation (mm)	ClimateNA v5.21	
PAS	Precipitation As Snow (mm)	ClimateNA v5.21	Precipitation as snow cover acts as a thermal barrier for overwintering (Marshall & Sinclair 2012), as well as a reservoir of water for spring and summer host plant growth, so we expect butterfly movement to be facilitated by precipitation as snow.

AHM	Annual Heat:Moisture index	ClimateNA v5.21	
SHM	Summer Heat:Moisture index	ClimateNA v5.21	
Land cover broad-scale	broad-scale land cover information (10 classifications)	AVHRR Land cover data: Palko *et al.* 1995	These species of swallowtail butterflies are generally open-habitat species, avoiding closed and heavily forested habitats (Sperling 1987), so we expect open habitat types to facilitate butterfly dispersal.
Land cover fine-scale	detailed land cover information (36 classifications), excludes SE Alberta	National Resource Canada, Earth Observation for Sustainable Development of Forests, Landcover Classification Scheme 2003: http://www.nrcan.gc.ca/forests/measuring-reporting/remote-sensing/13433	

Table 5.2 Significant resistance schemes from resistance scheme optimization for the *P. zelicaon* and hybrid scale and the Foothills region scale (FH). MSATs: microsatellite dataset; COI: *Cytochrome oxidase subunit I* dataset; Q: *Q*-distance (absolute distance matrix created from STRUCTURE results population averages). The variable "Euclidean" refers to log(geographic distance) in kilometers.

Genetic Measure	Variable	Mantel R^2	*P*-value
MSATs Nei's D	Euclidean	0.2112	0.001
	MAT, res9	0.3940	0.001
	EMT, res2	-0.2489	0.025
	Slope, res6	0.3157	0.018
MSATs G''_{ST}	Euclidean	0.3659	< 0.001
	MAT, res8	0.2073	0.037
	EMT, res2	-0.2193	0.012
MSATs Q	MAP, res8	0.3149	0.010
COI Jost's D	Euclidean	0.2205	< 0.001
	Land cover, res2	0.3472	0.006
FH MSATs Nei's D	Euclidean	0.2646	0.009
	MAT, res5	0.2722	0.011
	MAP, res6	0.2839	0.008
	EMT, res4	0.3013	0.007
	Slope, res1	0.2280	0.038
FH MSATs G''_{ST}	Euclidean	0.3443	0.002
	MAT, res5	0.2669	0.010
	MAP, res4	0.3497	0.004
	EMT, res5	0.2659	0.008
	Slope, res1	0.2299	0.024
FH MSATs Q	*No significant tests*		
FH COI Jost's D	Euclidean	0.2779	0.007
	Land cover, res5	-0.2824	0.035
	MAT, res2	0.2868	0.021
	MAP, res2	0.2808	0.008
	EMT, res6	0.2751	0.011
	Slope, res1	0.2705	0.010

Table 5.3 Results of multiple regression on distance matrices (MRDM) for the *P. zelicaon* and hybrid scale and the Foothills region scale (FH). MSATs: microsatellite dataset; COI: *Cytochrome oxidase subunit I* dataset; Q: *Q*-distance (absolute distance matrix created from STRUCTURE results population averages); VIF: variance inflation factor. The variable "Euclidean" refers to log(geographic distance) in kilometers.

Genetic Measure	Variable	β	*P*-value	VIF	Model R^2	Model *P*-value
MSATs Nei's D	Euclidean	0.0418	0.0003	1.01	0.2100	0.0011
	MAT, res9	0.0090	0.0029	2.70		
MSATs G''_{ST}	Euclidean	0.0455	0.0001	1.01	0.1554	0.0004
	MAT, res8	0.0036	0.0442	2.16		
MSATs Q	MAP, res8	0.0016	0.0044	2.58	0.1409	0.0065
	Slope, res2	-0.0016	0.0264	6.67		
COI Jost's D	Euclidean	0.0492	0.0005	1.01	0.2073	0.0001
	Land cover, res2	0.0492	0.0001	1.45		
FH MSATs Nei's D	EMT, res4	0.0056	0.0121	10.59	0.0916	0.0121
FH MSATs G''_{ST}	MAP, res4	0.0108	0.0043	6.81	0.1233	0.0043
FH MSATs Q	*no model supported*					
FH COI Jost's D	MAT, res2	0.0213	0.0074	3.69	0.1362	0.0074

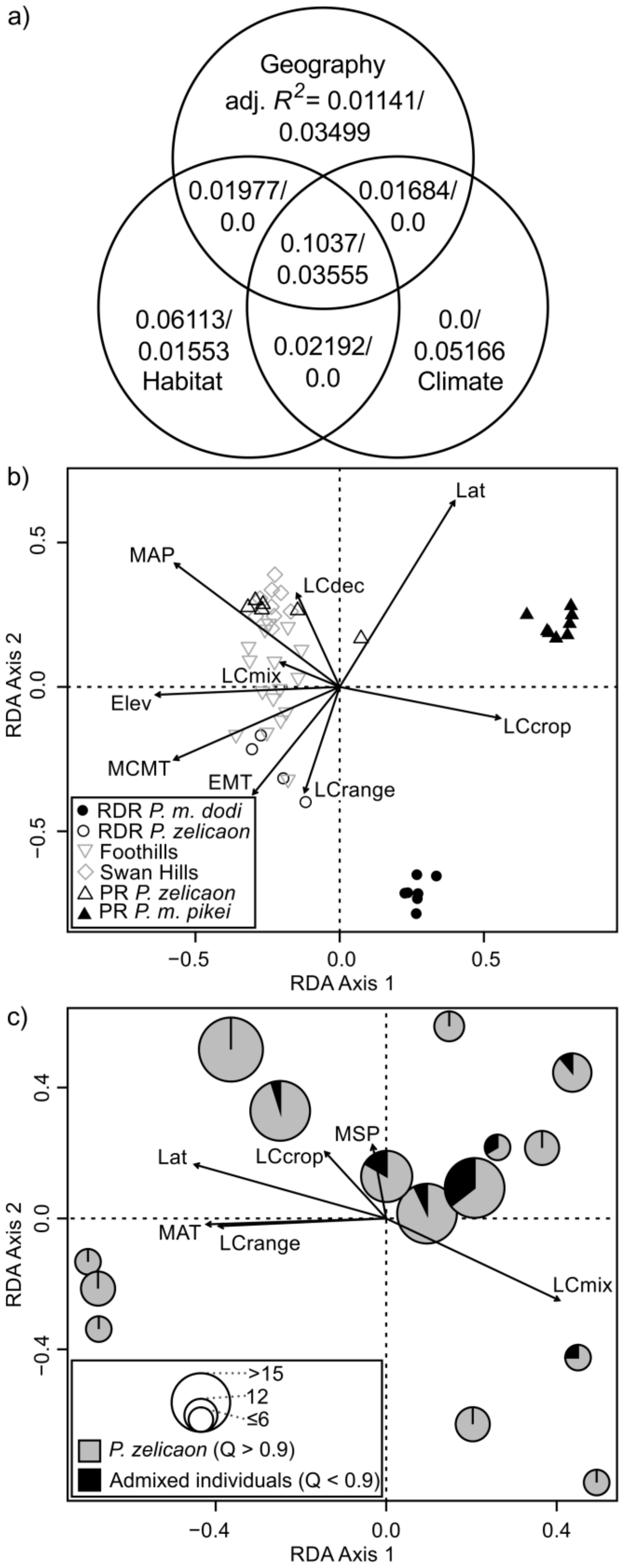

Figure 5.1 Results of redundancy analysis and variance partitioning of microsatellite data. (a) Adjusted R^2 values for the effects of variable categories (geography, climate, and habitat) on

Hellinger transformed allele counts (separate and combined effects), calculated with variance partitioning; number before slash is for all populations, and number after slash is for hybrid populations only. (b) Redundancy analysis of all populations with symbols demarcating regions as in (chapter 4). (c) Redundancy analysis of populations in the Foothills region. Populations are represented by pie charts (size denotes sample size of population) indicating the numbers of "pure" *P. zelicaon* individuals ($Q > 0.9$) and admixed individuals ($Q < 0.9$) based on the *P. zelicaon* genetic cluster from STRUCTURE at $k = 2$. Elev: elevation; EMT: extreme minimum temperature; Lat: latitude; LCcrop: cropland; LCdec: deciduous forest; LCmix: mixed forest; LCrange: rangeland; MAP: mean annual precipitation; MAT: mean annual temperature; MCMT: minimum coldest month temperature; MSP: mean summer precipitation.

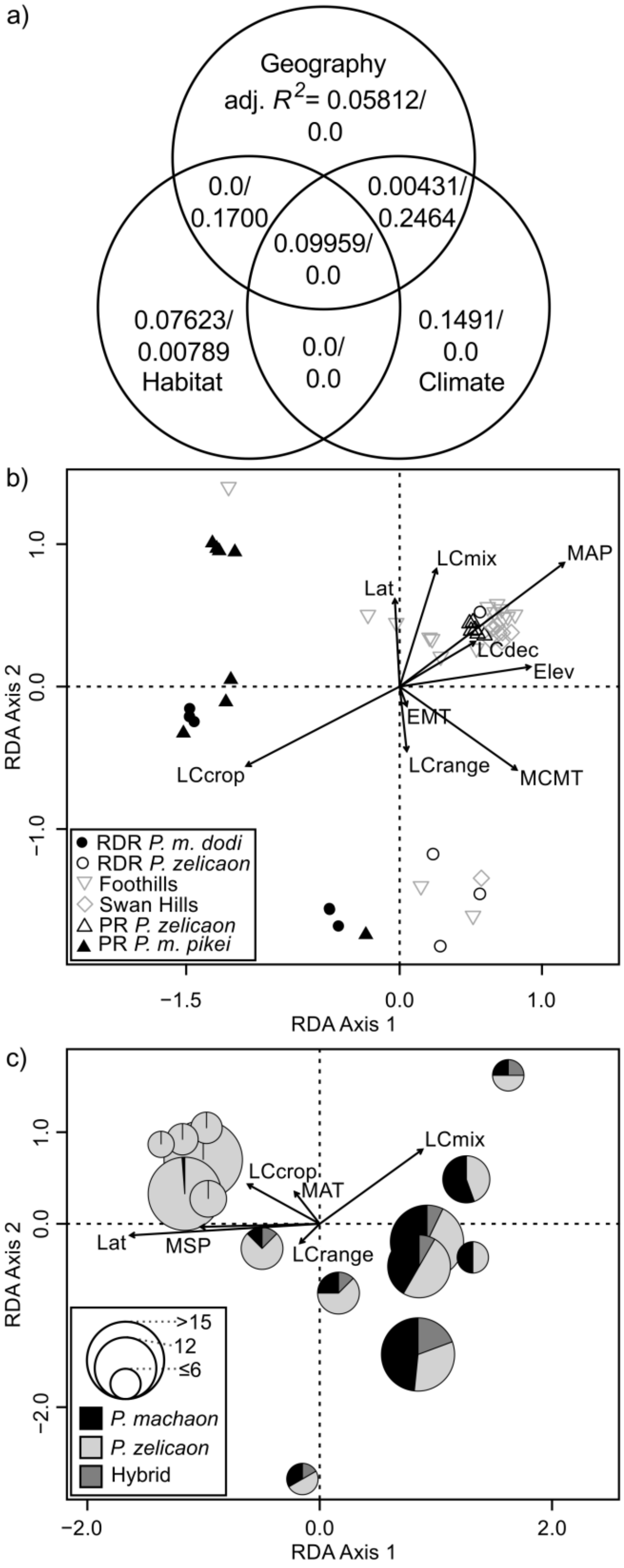

Figure 5.2 Results of distance-based redundancy analysis and variance partitioning for COI dataset. (a) Adjusted R^2 values for the effects of variable categories (geography, climate, and

habitat) on Jost's D genetic distance matrix for COI sequences (separate and combined effects), calculated with variance partitioning; number before slash is for all populations, and number after slash is for hybrid populations only. (b) Distance-based redundancy analysis of all populations with symbols demarcating regions as in (chapter 4). (c) Distance-based redundancy analysis of populations in the Foothills region. Populations are represented by pie charts (size denotes sample size of population) indicating the number of *P. machaon*, *P. zelicaon*, and hybrid type mtDNA haplotypes in each population. Some clusters of populations in (b) and (c) have been manually separated to clarify their placement. Elev: elevation; EMT: extreme minimum temperature; Lat: latitude; LCcrop: cropland; LCdec: deciduous forest; LCmix: mixed forest; LCrange: rangeland; MAP: mean annual precipitation; MAT: mean annual temperature; MCMT: minimum coldest month temperature; MSP: mean summer precipitation.

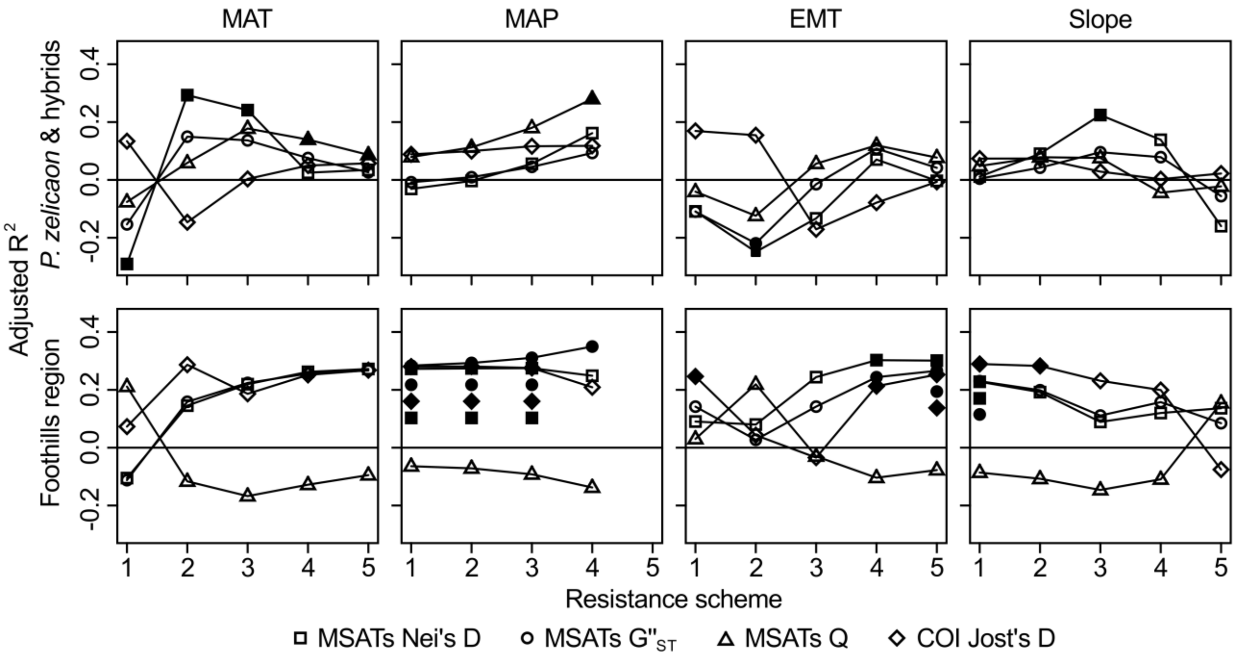

Figure 5.3 Correlations for "linear resistance schemes" for *P. zelicaon* and hybrid populations and populations in the Foothills region. Resistance scheme 1 corresponds to lower values having lower resistances for the variable in question, and resistance scheme 5 to higher values having lower resistances. Significant Mantel tests are indicated with filled symbols, and overlapping symbols have been spread out to display them more clearly. Missing symbols correspond to null surfaces, which were omitted. EMT: extreme minimum temperature; MAT: mean annual temperature; MAP: mean annual precipitation.

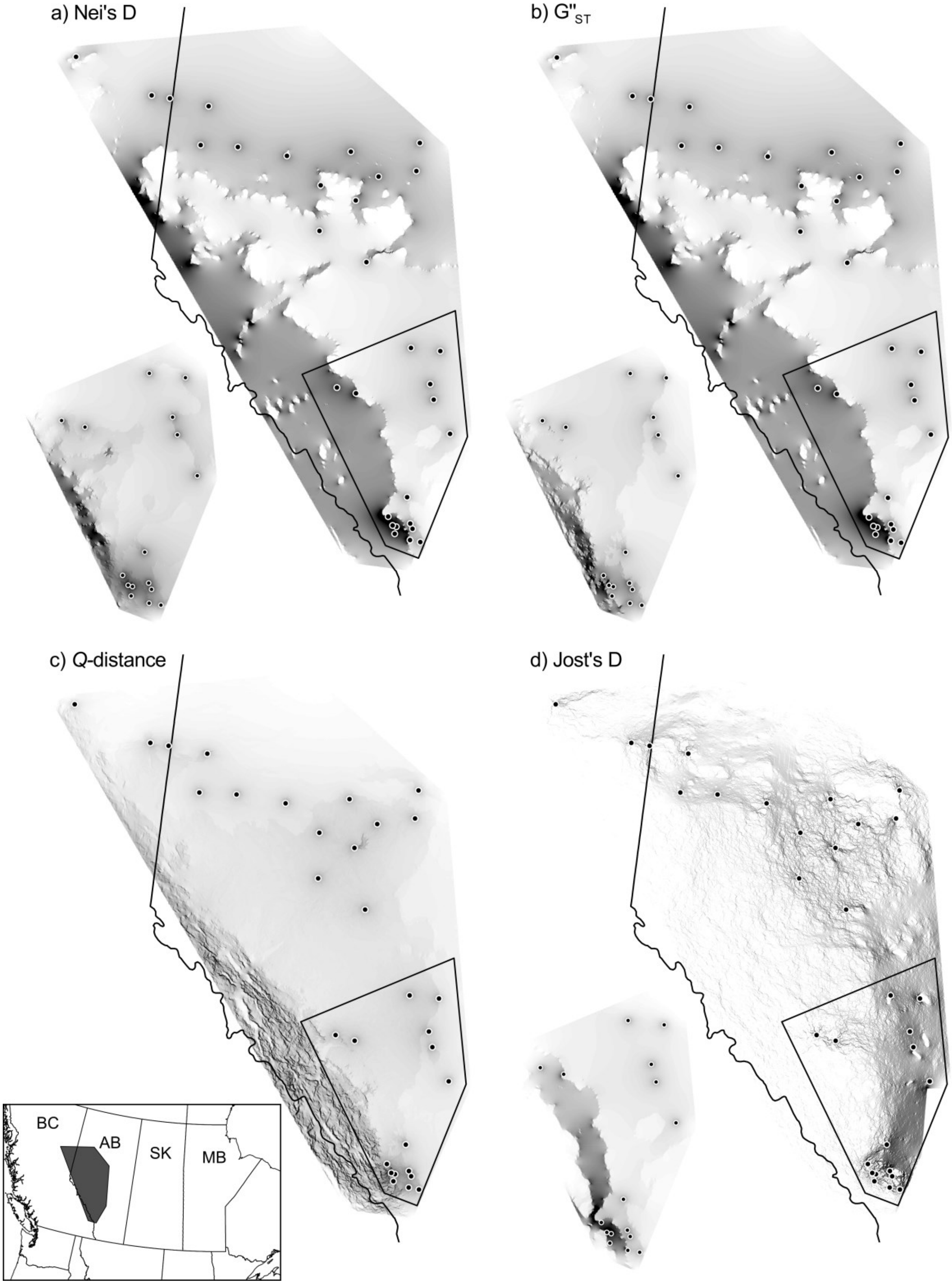
a) Nei's D
b) G"ST
c) Q-distance
d) Jost's D
BC
AB
SK
MB

Figure 5.4 Resistance surfaces of final cumulative models (see Table 5.3) supported by multiple regression on distance matrices (MRDM), for a) Nei's D, b) G''_{ST}, c) *Q*-distance, and d) Jost's D (COI). Darker coloration indicates lower resistance or higher connectivity. Population localities are indicated with black and white ringed points, and resistance surfaces for Foothills region scale are shown in the lower left hand corner of each panel (Note, no model supported for Foothill region scale of panel c). Extent of *P. zelicaon* and hybrids scale indicated in lower left hand corner, and extent of Foothills region scale indicated in each panel.

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Chapter 6

General conclusion

6.1 Thesis overview

In 1955 Sir Cyril Clarke and Philip Sheppard stated: "it is clear that the Machaon-group provides some of the most suitable material ever investigated in animals for studying the process of speciation in detail, taking into account genetic, ecological and behaviour differences as well as time" (Clarke & Sheppard 1955). Six decades later, our understanding of molecular biology, genetics, and speciation has changed immeasurably, but the *Papilio machaon* species complex continues to provide a rich system with which to expand our knowledge of evolutionary biology. In this thesis, I use a combination of systematics, population genetics, and spatial ecology to address three of the aspects of speciation identified by Clarke & Sheppard: genetics, ecology & behavior, and evolutionary time.

Species delimitation is fundamental for many disciplines of evolutionary biology, and increased use of genetic and genomic techniques in phylogenetics and systematics has revolutionized how species boundaries are determined (e.g. Fujita *et al.* 2012, Carstens *et al.* 2013). The use of multiple lines of evidence, or molecular markers from a genetic perspective, has ushered evolutionary biologists into the age of "integrative taxonomy" (Yeates *et al.* 2011). In **chapter 2,** I conducted the first taxonomically comprehensive review of studies that used multiple molecular markers to delimit closely related species of animals and fungi. My approach focused on closely related species, where species delimitation is often most difficult, to avoid inflating success rates by the inclusion of more divergent taxa (a phenomenon apparent in many early DNA barcoding studies: Hebert *et al.* 2003, Barrett & Hebert 2005, Janzen *et al*. 2005, but see Whitworth *et al.* 2007, Meier *et al.* 2006, Wiemers & Fiedler 2007). Four classes of genetic markers showed roughly equal, yet moderate, success (~70%) in delimiting closely related species, which supports the use of multiple genetic markers in species delimitation. Furthermore, power analysis showed that increasing the number of markers used to delimit species increased species delimitation success.

Contrary to theoretical expectation (Avise *et al.* 1987, Moritz & Cicero 2004, Meier 2008), we found no support for the expectation that increasing population or geographic sampling decreased delimitation success. Although this could be caused by biases of the review process or selective sweeps, it is clear that further work on generalizing the effects of intraspecific variability on species delimitation success is needed (e.g. Huemer *et al.* 2014). Perhaps more importantly however, this chapter emphasizes that species are taxonomic hypotheses, rather than hard and fast divisions of the natural world. Without thorough appreciation of this concept, our understanding of the complexity and subtlety of the biological world is threatened by a mental framework that strives for self-referential consistency rather than accuracy. The evolutionary phenomena that cause failure in DNA-based identification systems such as DNA barcoding, are the processes and scenarios that have the highest promise of increasing our overall knowledge of evolution.

Hybridization is one such phenomenon that poses problems for species delimitation. The reticulate nature of interbreeding between divergent populations, lineages, or species can homogenize genomes, but can also provide novel adaptive combinations that facilitate speciation (Rieseberg *et al.* 2003, Mallet 2007, Jiggins *et al*. 2008, Hedrick 2013). In **chapter 3**, I used morphology, ecological characteristics, mitochondrial DNA (mtDNA), and microsatellites to assess putative hybrid origins for several lineages of the *P. machaon* species group in North

America. This data was then used to distinguish between adaptive introgression and hybrid speciation for each of these lineages. Despite broad geographic separation, all four lineages shared identical *P. m. hudsonianus*-like mtDNA haplotypes, indicating similar maternal influence and Pleistocene origins. Morphology, ecology, and nuclear microsatellites showed variable parental characteristics across lineages, stressing the importance of using multiple lines of evidence when evaluating systematic questions. Only one of the lineages, *P. joanae*, had sufficient evidence (ecological separation from parental taxa) to define it as a hybrid species, which may have conservation ramifications (Schweitzer *et al.* 2011).

This research adds to the growing list of recognized hybrid taxa and to our understanding of hybridization's complex outcomes. The multifarious nature of hybrid lineages in the *P. machaon* group shares many characteristics with *Lycaeides* butterflies in western North America (Gompert *et al.* 2006, Gompert *et al.* 2014), and the repeated mitochondrial fixation across hybrid lineages (all from a single maternal donor lineage) is akin to *Lepus* hares in North America and Europe (Melo-Ferreira *et al.* 2012, Melo-Ferreira *et al.* 2014). The capacity of hybridization to facilitate speciation through adaptive introgression and hybrid speciation provides a novel lens with which to view biodiversity (Abbott *et al.* 2013). However, more research such as this, which differentiates between particular outcomes of hybridization, will be needed to fully appreciate this evolutionary process.

In **chapter 4**, I focused on comparisons among different assessments of hybrid interactions between *P. machaon* and *P. zelicaon* in southwest Alberta. Using mtDNA and microsatellites, and genome-wide single nucleotide polymorphisms (SNPs) for a subset of individuals, I used a population genetic approach to answer three main questions. First, were similar hybrid signatures present as compared to an assessment using morphology and allozymes

30 years ago (Sperling 1987, Sperling 1990)? Second, do different genetic markers indicate similar hybrid characteristics? And third, do different methods for hybrid identification or classification produce alternative interpretations of this hybrid interaction? Overall, genetic differentiation between *P. machaon* and *P. zelicaon* was high, but surprisingly, I observed far fewer hybrid individuals compared to previous studies. The number of hybrids identified, and the hybrid class of those individuals (F1, F2, backcross), depended on the genetic marker used. Through the use of simulated datasets based upon the empirical data, I tested the efficacy of the microsatellite and SNP datasets to identify and classify hybrid individuals; I found that genome-wide SNPs were more successful at both tasks, corroborating previous findings (e.g. Fitzpatrick 2012). These results stress the importance of using multiple data types when interpreting hybrid zone structure, particularly when classifying hybrid individuals. Although fewer hybrids were observed as compared to Sperling (1987), the informativeness of different genetic markers used in each of these studies (neutral versus adaptive) are likely to be at least partially responsible. Intermediate specimens have been recorded since the early 1900's, and so this hybrid interaction remains likely to be a stable hybrid swarm.

The dynamics of hybrid zones are often influenced by environmental or landscape-based factors, but studies assessing the effects of landscape features on hybridization often do so only with simplified associations between landscape and hybrid occurrence (Fitzpatrick & Shaffer 2007, Pfennig 2007, Larson *et al.* 2013). No studies have used raster-based landscape genetic analyses (e.g. multiple regression on distance matrices) to investigate gene flow in a hybrid system. In **chapter 5**, I extended a landscape genetic approach to the hybrid zone between *P. machaon* and *P. zelicaon* in southwest Alberta. I used raster-based landscape genetic analyses and redundancy analysis to investigate the effect of environmental and landscape variables on genetic differentiation in the system. I also explored the effect of various genetic distance measures and spatial scales on these analyses. Using redundancy analysis, cropland explained the most variability between *P. machaon* and *P. zelicaon*, acting as a surrogate variable for the arid river valley habitats of *P. machaon* that are adjacent to croplands. Other land cover and climatic variables (particularly mixed forest) explained some genetic differentiation between hybrid and *P. zelicaon* populations, but to a lesser degree. Raster-based resistance scheme optimization and multiple regression on distance matrices produced variable results that were dependent on the genetic distance measure used and spatial scale, a phenomenon already observed elsewhere (Storfer *et al.* 2010, Pérez-Espona *et al.* 2012, Keller *et al.* 2013, Zancolli *et al.* 2014). Overall, I observed high linear connectivity along the east slope of the Rocky Mountains, and more patchy connectivity away from the Rocky Mountains.

This extension of landscape genetic methods to multi-species systems is an emerging trend (James *et al.* 2011, Hand *et al.* 2015), but this is the first use of raster-based landscape genetic analyses in a hybrid system. The conceptual shift from considering gene flow within species to gene flow between species requires more than just applying new analyses to multi-species systems. Here, I used hierarchical spatial scales to address hybrid zone gene flow (in an area of predominately *P. zelicaon*) while avoiding bias from much greater genetic differentiation between *P. machaon* and *P. zelicaon*. However, results of raster-based analyses with different genetic measures were highly variable, which speaks to the need for general consensus on the appropriateness of various measures of genetic differentiation in landscape genetics.

6.2 Future directions

This thesis addresses many aspects of the complex evolutionary history of the *P. machaon* species group. However, much remains to be learned from these butterflies, and I have several additional projects already underway that were born during the development of this thesis. Of most immediate interest is to propel the research questions fully into the "genomic era". I began doing this in chapter 4, with the genome-wide SNP dataset, which unfortunately was only available for a subset of individuals. Genotyping by sequencing (GBS) data has already been collected for a follow-up of chapter 3, which will investigate the genomic characteristics of the four hybrid lineages considered in that chapter. I have also expanded my sampling efforts from that chapter, and have broader geographic coverage in North America (and South America for *P. polyxenes*), as well as many subspecies of *P. machaon* from Europe. Given the high degree of retained ancestral polymorphism observed in mtDNA of *P. machaon* (identical haplotypes found across its Holarctic range), this will provide a rich foundation with which to compare the fixed *machaon*-like mtDNA haplotypes found in the hybrid lineages to their overall genomic characteristics. Expanding the genomic dataset of chapter 4 would be another worthwhile endeavor, as it would allow genomic extensions of the landscape genetics approaches from chapter 5, and provide an opportunity to zero in on the genes that are responsible for maintaining species boundaries in the group.

Exploration of the genomic architecture of speciation in the *P. machaon* group should also provide insights. A genomic reference assembly for *P. machaon* was recently published (September 2015: Li *et al.* 2015), but was based on a linkage map for a closely related species, *P. xuthus*, which was used throughout this thesis as an outgroup. This genomic assembly is still in its infancy, with over 63 thousand scaffolds (Li *et al.* 2015). In collaboration with a skilled amateur Lepidopterist in Denmark, Sune Hauch, I also have a GBS dataset for several hundred individuals of bidirectional second-generation backcross linkage mapping families (between *P. machaon* and *P. polyxenes*). With this dataset, I plan to construct a high-density linkage map, to add to the genomic resources available for the species group. In addition to facilitating genome reference assemblies, this resource will also allow me to build on seminal research in the *P. machaon* species group by Clarke & Sheppard (1955) to investigate the genomic regions responsible for various morphological characters, including black versus yellow wing coloration, hindwing pupil shape, and orange patches in the postmedian area of the ventral hind wing. These characters are frequently used for species delimitation in the group, but are highly variable within species and geographic races (Scott 1986, Sperling 1987). This project will also use geometric morphometrics, as compared to the qualitative morphological measurements conducted by Clarke & Sheppard (1955), and will ideally link genomic characteristics to quantitative morphological measurements.

The evolutionary complexity of the *P. machaon* group provides a rich foundation to study speciation, hybridization, and systematics, and the past sixty years of research since Clarke & Sheppard's work stands as a testimonial to that. I am sure many of the lepidopterists cited in this thesis would agree that these butterflies also have a charismatic charm that is found in few other animal systems. This combination furthers the potential of this system and, with continued advances in research approaches, the *P. machaon* group will surely continue to foster evolutionary theory in the coming years.

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Appendix 2.1 Methods used in literature survey and analysis

Literature survey

Our literature survey was designed to assess the success of multilocus species delimitation of closely related animal and fungal species. Web of Science searches were conducted (Table S1), and preliminary screening of literature focused on studies that used multiple independent genetic markers to delimit closely related species. Any studies published from 1990 to February 2011 were used. General keyword searches (using "nuclear" as a base keyword, as it is often featured in any study using multiple molecular markers for species delimitation) were followed by journal-specific searches to achieve comprehensive coverage (Table S1). From this original pool of potentially appropriate literature, studies were subsequently screened based on a set of predefined criteria.

First, only studies concerned with delimitation of closely related species were used. Research dealing with genus-level relationships or intraspecific variation (without distinguishing species) was excluded. Determining what constituted "closely related species" was challenging. In the strictest sense, sister-species would be the ideal comparison. However, strict application of this definition requires that all other species in a group are known and have been examined, which was often not the case. Therefore, we defined closely related species as those that have been shown through multiple lines of evidence (morphology, host plant, geographic range, molecular data, etc.) to be "good species" (taxonomically distinct) within a genus. Some fungal species belong to different genera, but are considered to be in the same clade. Studies that focused on taxonomically undescribed or cryptic taxa were accepted as long as the authors unambiguously defined the taxa in question.

Second, we included only studies that used multiple independent genetic markers to delimit species. Independent loci ensure that the markers in question are not inherited as a single lineage block (e.g. two mitochondrial loci would show the same evolutionary tract). Studies using other methods for species delimitation (morphology, geographic range, ecology, etc.) were considered acceptable providing they used a minimum of two molecular markers.

We also required that multiple individuals were sampled for each species, in an effort to evaluate intraspecific variation. We set a minimum of five individuals per species for this meta-analysis, and all species being compared had to meet this minimum number, to reduce bias in study selection and ease the determination of phylogenetic relationships. For example, if two of three species being delimited had adequate sampling, but one did not, the study was rejected. Poorly sampled outgroup taxa were an exception; if the identified outgroup(s) of a study had fewer than five individuals, the study could still be accepted. Additionally, many studies that focused on a single pair of taxa often include both “true” outgroups (which the authors call outgroups), as well as several individuals of another closely related species. If this species was inadequately sampled, but was supplementary to the question at hand (distinguishing the original pair of taxa), the study could still be accepted, with the inadequately sampled taxa treated as an outgroup. This minimum sampling rule had to also apply to the multiple genes in a study. If an individual marker was available for less than five individuals of a species (as is often the case when sequencing both mitochondrial and nuclear genes), the study was rejected. If a gene subsampled fewer than five individuals, but the study still used two or more independent genes that met the sampling criteria, the study was considered acceptable, with the omission of the under-sampled gene.

Finally, only unique studies (not a duplicate of an later study with fewer markers) were used, and these had to provide data from all markers individually. If either haplotype or gene tree information were missing for a locus, then the study was rejected. This was most commonly encountered where authors provided only combined phylogenetic results. If these situations arose, but access was provided to an aligned data file through a website such as TreeBase, basic phylogenetic analysis were conducted to assess haplotype fixation and phylogenetic congruence.

Once we established the subset of studies that met all the criteria above we evaluated the extent of geographic sampling of the species. A combination of techniques was used to approximate the global distribution (including introductions) of each species. If the authors of the original studies provided the distribution, even as an approximation, this was used. If the authors did not mention the full distribution, Google and Google scholar searches of the species name and "distribution" or "range", were conducted (For example: *Genus species* distribution). Depending on the organism group, additional online databases were useful in this effort, and were added to search terms (e.g. BugGuide: Van Dyk 2011; World Asteroidea Database: Mah 2009). Additionally, the term "review" was added to searches to target recent reviews of genera, when other methods were unsuccessful in obtaining a distribution. Once estimated, global distributions were categorized into the following groups: A) total distribution is <100 km in diameter; B) distribution is <1000 km in diameter; C) distribution is between 1000 km and continent wide (Americas, Eurasia, Africa, Australia/Australasia, and Antarctica), or for marine species, distribution is between 1000 and 5000 km in diameter; and D) distribution spans more than one continent, or for marine species, distribution is >5000 km in diameter.

Upon classification of the approximate global distribution, the extent of sampling was calculated based on how many "quarters" of the whole distribution were sampled (i.e. ≤ 25%, ≤

50%, ≤ 75%, or ≤ 100% of the species' world distribution). This approximation was done by one of the authors (JRD). If a distribution could not be determined, such as in poorly known taxa, the study was omitted from this analysis. This is a coarse estimate that is dependent on the ease of sampling of the organisms and their relative prevalence in the literature and online databases (and thus the amount of knowledge known about them generally), but also the subjectivity of the data collector in estimating the extent of sampling. This method does, however, distinguish between: 1) studies that attempted to capture the complete geographic variation within the species, to those that did not, and 2) species that are widespread to those that are geographically restricted.

Details of FI and CGI are discussed in the methods. In calculating the CGI we relied on the species boundaries (definitions) preferred by the authors to score the phylogenetic or clustering relationships. Although the use of multiple species concepts may be contentious (see DeQueiroz 2007), we believe it is necessary for a comprehensive review methodology. Ultimately, the authors researching these organisms are likely to be the most knowledgeable experts for each group, and we trust their judgment in assessing "a priori" relationships. Furthermore, the sheer number of species included in these analyses includes ones originally described using morphology, molecular, and other methods that inherently involve different species concepts. We do not discuss these issues in detail, but acknowledge that there are substantial complexities surrounding species concepts and use the authors' preferred species limits as the best a priori integration of these issues.

Multilocus power analysis

All accepted studies were considered for inclusion into the multilocus power analysis (see Roe *et al.* 2010). Due to the nature of these analyses (see methods), access to the aligned data files (DNA sequences) was necessary. Though providing GenBank accession numbers is currently the normal procedure for molecular publications, and it is possible to use individual sequence files for this analysis, there are several reasons why they are ill suited in this regard. Most importantly, alignment of all sequences (and all loci) in a study would be required after the GenBank files were retrieved. In this review, studies sampled up to 320 specimens per species, 12 species, and 27 loci. Considering the expertise required in aligning many types of genes, coming to a consensus regarding gaps, indels, and coding vs. non-coding sequence (which was highly varied among the studies in this review), and the possible introduction of errors occurring during the renaming of accession-numbered sequences with taxa names, we felt that this step would be unwise. Instead, we obtained data files in two ways: 1) We searched and retrieved alignments from the online database TreeBASE; and 2) we contacted authors, whose studies included in our literature review used two or more sequence-based data sources, and requested the aligned data files. From aligned data files in which taxa names and general organization were comprehensible, we concatenated all loci into a single nexus formatted file and conducted the power analysis (see methods). For loci that expressed heterozygote genotypes (i.e. phased sequence data), ambiguity codes were used to allow concatenation with single allele loci. Due to the nature of the power analyses, data files using haplotypes as taxa with no reference to individual specimens' multilocus genotypes were unusable. Additionally, due to the shear number of possible combinations of, for instance, two loci in a study using ten loci (45 combinations in this example), we also omitted studies that included greater than five loci (there were only a small number of studies that included more than five loci). Finally, though these analyses are based upon those conducted in Roe *et al.* (2010), not all studies used therein met our strict screening methods.

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Appendix 2.2 Web of Science search term combinations used for scanning the literature to assess multilocus species delimitation of closely related species. Journal names were specified as "Publication Name" and keywords were specified as "Topic" in Web of Science search algorithm. * refers to a wildcard in the search criteria.

Keyword searches:	**Keywords**		
	nuclear AND delimitation		
	nuclear AND delimit		
	nuclear AND "species complex"		
Journal-specific searches:	**Journal**		**Keyword**
	Molecular Phylogenetics and Evolution	AND	nuclear
	Molecular Ecology	AND	nuclear
	Molecular Ecology	AND	autosomal
	Systematic Biology	AND	nuclear
	Evolution	AND	nuclear
	Evolution*	AND	multilocus
	Canadian Journal of Zoology	AND	nuclear
	Canadian Journal of Zoology	AND	phylo*
	Canadian Journal of Microbiology	AND	nuclear
	Canadian Journal of Microbiology	AND	phylo*
	Canadian Journal of Fisheries	AND	nuclear
	BMC Evolutionary Biology	AND	nuclear
	Heredity	AND	nuclear
	Conservation Genetics	AND	nuclear
	Journal of Evolutionary Biology	AND	nuclear
	Auk	AND	nuclear
	Oikos	AND	nuclear
	Journal of Avian Biology	AND	nuclear
	Ibis	AND	nuclear
	Condor	AND	nuclear
	Journal of Biogeography	AND	nuclear
	Molecular Biology and Evolution	AND	nuclear
	Cladistics	AND	nuclear
	Zoological Journal of the Linnean Society	AND	nuclear
	Proceedings*	AND	nuclear
	Proceedings*	AND	multilocus
	Ento*	AND	nuclear
	Annals of the Entomological Society of America	AND	nuclear
	Studies in Mycology	AND	nuclear
	Phytopathology	AND	nuclear
	Myco*	AND	nuclear

Appendix 2.3 Summary of rejected studies, grouped by reasons for rejection

	Accepted	**Unacceptable Studies**						**Total**
	studies	Intraspecific	Low sample size*	Missing data †	Supraspecific‡	Duplicate	Undefined taxa₷	**examined**
Hexapoda	31	2	39	27	2	4	8	113
Misc. Invertebrates	13	5	40	20	2	0	9	89
Fishes	11	1	15	11	1	1	7	47
Amphibians	12	0	7	3	1	0	2	25
Reptiles	5	1	18	24	0	1	4	53
Birds	7	5	11	9	2	0	1	35
Mammals	13	1	13	10	0	4	0	41
Fungi	9	0	11	2	0	0	0	22
Total	101	15	154	106	8	10	31	425

*Low sample size had <5 individuals sampled per species per locus (if >2 loci were used, any loci sampled in <5 individuals were excluded). †Either haplotype information or gene tree information were missing for a locus. ‡Study did not deal with species limits. ₷Taxa were ambiguous (but cryptic or undescribed taxa were included if the authors clearly defined them in the study).

Appendix 2.4 Characteristics of genetic markers used for delimitation of closely related species, grouped by clade or grade/cluster, with n representing the number of studies examined. A) All organismal groups; B) Hexapoda only.

A)

Organismal Group	**Location**	**Total**	**Fixed**	**Shared**	**RM**	**PA/PO**	**C**	**NC**	**NA**	**FI**	**CGI**
Hexapoda	mtDNA	28	17	11	9	7	1	0	11	0.61	0.59
n=31	rDNA	13	10	3	1	2	5	2	3	0.77	0.60
	autosomal	93	67	26	7	7	27	25	27	0.72	0.51
	sex-linked	27	25	2	4	0	11	10	2	0.93	0.60
	anonymous	6	0	0	0	0	5	1	0	NA	0.83
Misc. Invertebrates	mtDNA	13	9	4	5	4	0	0	4	0.69	0.56
n=13	rDNA	8	7	1	5	2	0	0	1	0.88	0.71
	autosomal	8	2	6	2	0	0	0	6	0.25	1.00
	sex-linked	*1*	*0*	*1*	*0*	*0*	*0*	*0*	*1*	*0.00*	*0.00*
Fishes	mtDNA	11	7	4	5	2	0	0	4	0.64	0.71
n=11	*rDNA*	*2*	*2*	*0*	*0*	*2*	*0*	*0*	*0*	*1.00*	*0.00*
	autosomal	7	2	5	0	2	0	0	5	0.29	0.00
	allozymes	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*NA*	*1.00*
	anonymous	5	0	0	0	0	4	1	0	NA	0.80
Amphibians	mtDNA	12	11	1	7	4	0	0	1	0.92	0.64
n=12	autosomal	21	16	5	5	11	0	0	5	0.76	0.31
	allozymes	*2*	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*NA*	*1.00*
	anonymous	*1*	*0*	*1*	*0*	*0*	*0*	*0*	*1*	*NA*	*0.00*
Reptiles	mtDNA	5	5	0	2	3	0	0	0	1.00	0.40
n=5	autosomal	13	5	8	1	4	0	0	8	0.38	0.20
	anonymous	*1*	*1*	*0*	*1*	*0*	*0*	*0*	*0*	*1.00*	*1.00*
Birds	mtDNA	7	4	3	4	0	0	0	3	0.57	1.00
n=7	autosomal	6	1	5	1	0	0	0	5	0.17	0.00
	sex-linked	6	2	4	1	1	0	0	4	0.33	0.50
	anonymous	*3*	*0*	*0*	*0*	*0*	*3*	*0*	*0*	*NA*	*1.00*
Mammals	mtDNA	12	9	3	3	4	1	1	3	0.75	0.44
n=13	*rDNA*	*1*	*0*	*1*	*0*	*0*	*1*	*0*	*0*	*0.00*	*1.00*
	autosomal	15	11	4	7	0	0	4	4	0.73	0.64
	sex-linked*	8	4	3	0	0	3	2	3	0.50	0.75
	allozyme	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*NA*	*1.00*
	anonymous	8	0	0	0	0	5	3	0	NA	0.63
Fungi	*mtDNA*	*2*	*2*	*0*	*0*	*2*	*0*	*0*	*0*	*1.00*	*0.00*
n=9	rDNA	8	4	4	2	0	1	1	4	0.50	0.75
	autosomal	22	18	4	11	4	3	0	4	0.82	0.78
	anonymous	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*NA*	*1.00*
Total	mtDNA	90	64	26	35	26	2	1	26	0.71	0.58
n=101	rDNA	31	23	8	8	6	6	3	8	0.74	0.61
	autosomal	185	122	63	34	28	30	29	64	0.66	0.52
	sex-linked	42	31	10	5	1	14	12	10	0.74	0.61

	allozymes	*4*	*0*	*0*	*1*	*0*	*3*	*0*	*0*	*NA*	*1.00*
	anonymous	25	1	1	1	0	18	5	1	NA	0.76

B) **Order**	**Location**	**Total**	**Fixed**	**Shared**	**RM**	**PA/PO**	**C**	**NC**	**NA**	**FI**	**CGI**
Lepidoptera	mtDNA	14	10	4	6	4	0	0	4	0.71	0.60
n=13	*rDNA*	*1*	*0*	*1*	*0*	*0*	*0*	*0*	*1*	*0.00*	*0.00*
	autosomal	25	14	11	4	4	0	6	11	0.56	0.29
	ChrX	3	3	0	3	0	0	0	0	1.00	1.00
	ChrZ	2	2	0	0	0	0	2	0	1.00	0.00
	anonymous	*4*	*0*	*0*	*0*	*0*	*4*	*0*	*0*	*NA*	*1.00*
Diptera	mtDNA	6	3	3	0	2	1	0	3	0.50	0.33
n=10	rDNA	8	7	1	1	0	5	1	1	0.88	0.86
	autosomal	60	48	12	0	1	27	19	13	0.80	0.56
	ChrX	20	18	2	1	0	9	8	2	0.90	0.56
	ChrY	2	2	0	0	0	2	0	0	1.00	1.00
Other**	mtDNA	8	4	4	3	1	0	0	4	0.50	0.75
n=8	*rDNA*	*4*	*3*	*1*	*0*	*2*	*0*	*1*	*1*	*0.75*	*0.00*
	autosomal	8	5	3	3	2	0	0	3	0.63	0.60
	anonymous	*2*	*0*	*0*	*0*	*0*	*1*	*1*	*0*	*NA*	*0.50*

Total (TOTAL) number of loci used for each marker class/practical group and the number of fixed (FIXED) and shared (SHARED) haplotypes. Relationship between species: for phylogenetic analyses RM, reciprocal monophyly; PA/PO, paraphyly or polyphyly; for distance-based analyses C, congruence, NC, noncongruence; NA, not applicable (when shared haplotypes were present). FI, fixation index; CGI, congruence index. *Includes one sex-linked microsatellite. **Other includes orders Coleoptera, Hemiptera, and Hymenoptera (n = four, two, and two, respectively). Markers groups with <5 loci (for a taxonomic group) denoted with italics.

Appendix 2.5 Accepted studies with corresponding data, sorted by study and locus. Literature survey methods are described in Methods. Haplotype/allele fixation (or polymorphism data in round brackets) were measured per locus, as well as the congruence of those haplotypes based on the type of analysis used (phylogeny or distance-based). Phylogenetic-based (parsimony, Bayesian, or likelihood) congruence was characterized as: RM=reciprocal monophyly, M=monophyly, or PA/PO=paraphyly/polyphyly, and clustering methods (neighbor-joining, UPGMA, or other distance-based approaches) were scored as either: C=congruent, or NC=noncongruent. Though initial congruence records may contain multiple states, i.e. M+PA, for summarization into Table S3 any non-RM relationship was simplified as PA/PO.

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
HEXAPODA: LEPIDOPTERA					
Bull *et al.* 2006 *Heliconius cydno* x *H. melpomene*					
mtDNA	COI+COII	16	16	0	RM
autosomal	Mpi	33	31	2	-
	ci	39	39	0	PO
ChrX	Tpi	29	29	0	RM
Elias *et al.* 2007 *Mechanitis "mazaeus"* x *M. mazaeus* x *M. lysimnia* x *M. polymnia*					
mtDNA	COI-COII	11	11	0	PA/PO
autosomal	EF1A	16	16	0	PA/PO
Elias *et al.* 2007 *Hypothyris anastasia* x *H. euclea* x *H. fluonia* x *H. mamercus* x *H. moebiusi* x *H. semifulva*					
mtDNA	COI-COII	15	15	0	RM
autosomal	EF1A	16	16	0	RM
Elias *et al.* 2007 *Ithomia agnosia* x *I. amarilla* x *I. salapia*					
mtDNA	COI-COII	8	8	0	RM
autosomal	EF1A	8	8	0	RM
Elias *et al.* 2007 *Melinaea marsaeus* x *M. menophilus* x *M. satevis*					
mtDNA	COI-COII	7	7	0	PA/PO
autosomal	EF1A	7	7	0	PA/PO
Elkington *et al.* 2010 *Operophtera brumata* x *O. bruceata*					
mtDNA	COI	6	6	0	RM
autosomal	G6PD	5	5	0	RM
Gompert *et al.* 2006 *Lycaeidies idas* x *L. melissa* x *L. "alpine taxa"*					

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
mtDNA	COI+COII	14	14	0	M+PA+PA
autosomal	Nuc1	22	16	6	-
	Nuc3	18	18	0	NC
	EF1A	9	6	3	-
anonymous	Msat[d]	3	-	-	C
	AFLP	128	-	-	C
Kronforst *et al.* 2006 ***Heliconius cydno* x *H. melpomene* x *H. pachinus***					
mtDNA	COI+COII	106	80	26	-
Chr1[e]	EF1A	15	15	0	NC
	ptc	18	18	0	NC
Chr2	wg	24	24	0	NC
Chr3	cn	15	10	5	-
	st	25	16	9	-
	w	12	10	2	-
Chr4	ci	25	24	1	-
Chr5	sd	24	19	5	-
Chr6	DII	67	67	0	NC
	en	14	14	0	NC
	inv	26	18	8	-
Chr7	Mpi	30	22	8	-
ChrZ	Tpi	32	32	0	NC
	ap	23	23	0	NC
anonymous	AFLP	(657)	-	-	C
Narita *et al.* 2006 ***Eurema hecabe "Y type"* x *E. hecabe "B type"***					
mtDNA	ND5	10	8	2	-
	16S (*Wolbachia* mediation)	6	4	2	-
autosomal	EF1a	6	6	0	RM
ChrX	Tpi	42	42	0	RM
Prudic *et al.* 2008 ***Adelpha californica* x *A. eulalia* x *A. bredowii***					
mtDNA	COII	24	24	0	RM
ChrX	Tpi	7	7	0	RM
Roe & Sperling 2007 ***Dioryctria reniculelloides* x *D. pseudotsugella***					
mtDNA	COI	21	21	0	M+PA
rDNA	ITS2	4	3	1	-
autosomal	EF1A	4	3	1	-
Salvato *et al.* 2002 ***Thaumetopoea pityocampa* x *T. wilkinsoni***					
mtDNA	COI+COII	10	10	0	RM
anonymous	AFLP	183	-	-	C
Schoville *et al.* 2011 ***Colias meadii* x *C. behrii***					
mtDNA	COI	5	3	2	-
autosomal	EF1A	13	13	0	M+PA/PO

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
HEXAPODA: DIPTERA					
Bachtrog *et al.* 2006	***Drosophila yakuba* x *D. santomea* x *D. teissieri***				
mtDNA	COII+ND5	32	29	3	-
ChrX	4E	35	35	0	C
	6C	43	43	0	C
	8A	46	46	0	C
	10B	46	46	0	C
	11A	24	24	0	C
	12C	39	39	0	C
ChrY	kl-2 intron, kl-2, kl-3 5’, kl-3 intron 3’, ory-intron&exon	4	4	0	C
Besansky *et al.* 2003	***Anopheles gambiae* x *A. arabiensis***				
Chr2	tox	27(30)	25(0)	2(30)	-
Chr3	G6pd	23(8)	21(0)	2(8)	-
	xdh	24(11)	24(0)	0(11)	PO
ChrX	white	32(14)	32(8)	0(6)	RM
Hasan *et al.* 2008	***Anopheles farauti s.s.* x *A. irenicus***				
mtDNA	COII	39	39	0	PA+M
rDNA	ITS2	2	2	0	RM
Hemmerter *et al.* 2009	***Culex annulirostris* x *C. sp. "PNG"* x *C. palpalis***				
mtDNA	COI	51	51	0	PA+M+M
autosomal	ace-2	45	45	0	NC
Kliman *et al.* 2000	***Drosophila simulas* x *D. sechelia* x *D. mauratiana***				
mtDNA	ND5	5	5	0	C
Chr3	hb	17	17	0	NC
ChrX	per	16	16	0	NC
	yp2	12	12	0	NC
	z	10	10	0	NC
	ase	6	6	0	NC
	Sxl	33	33	0	NC
Llopart *et al.* 2005	***Drosophila yakuba* x *D. santomea***				
mtDNA	ND4-ND5	7 (12)	5 (0)	5 (2)	-
Chr1	y	1 (6)	0 (0)	1 (0)	-
	su(f)	5 (8)	5 (7)	0 (0)	C
	rux	15 (69)	15 (22)	0 (3)	C
	per	5 (31)	5 (13)	0 (0)	C
	sog	15 (41)	15 (1)	0 (2)	C
	bnb	13 (55)	13 (2)	0 (5)	C
	Hex-A	6 (15)	6 (3)	0 (0)	C
Chr2	l(2)gl	10 (5)	10 (1)	0 (0)	NC
	His3	2 (9)	2 (6)	0 (0)	C

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
	Kr	7 (9)	7 (6)	0 (0)	C
	Rad1	11 (25)	11 (1)	0 (2)	C
	RpL27A	13 (41)	13 (2)	0 (0)	C
	salr	15 (37)	15 (0)	0 (10)	NC
	Rep4	10 (58)	10 (4)	0 (1)	C
	Sara	13 (34)	13 (2)	0 (0)	C
	Hex-C	15 (32)	15 (0)	0 (2)	C
	Ngp	12 (13)	12 (3)	0 (0)	C
Chr3	Lsp1-≥	3 (11)	3 (4)	0 (0)	C
	Ssl1	6 (9)	6 (6)	0 (0)	C
	krz	3 (5)	3 (3)	0 (0)	C
	sfl	15 (67)	15 (2)	0 (12)	C
	Est6	16 (63)	16 (5)	0 (5)	C
	Xdh	11 (54)	11 (1)	0 (3)	C
	AP-50	14 (61)	14 (4)	0 (7)	C
	Mlc	12 (18)	12 (3)	0 (2)	C
ChrY	Dhc-Yh3 CG17629 Pp1Y1	6 (13)	6 (8)	0 (0)	C
Machado & Hey 2003 ***Drosophila pseudoobscura*** **x** ***D. persimilis*** **x** ***D. p. bogotana***					
mtDNA	COI ND4-ND5	31	30	1	-
Chr2	2001	42	42	0	NC
	2002	37	37	0	NC
	bcd	43	43	0	NC
	rh1	32	32	0	NC
	2003	26	23	3	-
Chr3	3002	36	36	0	NC
Chr4	4002	31	31	0	NC
	4003	41	41	0	NC
	Adh	33	33	0	NC
Chr5	ey	4	3	1	-
ChrXL	X008	37	37	0	C
	per	28	28	0	NC
ChrXR	X009	31	31	0	NC
	X010	16	15	1	-
	Hsp82	25	25	0	NC
Mazzoni ***et al.*** **2008** ***Lutzomyia intermedia*** **x** ***L. whitmani***					
rDNA	Rp49	15	15	0	C+NC
	Rp17A	13	12	1	-
	RpL36	18	18	0	C
	RpS19a	23	23	0	C
autosomal	CaID	20	20	0	NC
	cac	14	13	1	-
	per	20	20	0	NC
	Tfl1A-L	23	23	0	NC
	up	17	17	0	NC
	zetacop	25	23	2	-

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
Rona *et al.* 2010	***Anopheles cruzii "Itaparica"* x *A. cruzii "Florianopolis"***				
rDNA	Rp49	17	17	0	C
	Rp52	19	19	0	C
	RpS29	22	22	0	C
autosomal	timeless	33	33	0	C
	Clock	12	12	0	C
	cycle	16	16	0	C
Turner *et al.* 2005	***Anopheles gambiae "M form"* x *"S form"***				
Chr2L	LIM	7 (9)	7 (4)	0 (0)	C
	Ion Channel	2 (3)	2 (3)	0 (0)	C
	Subtilase	46 (26)	43 (0)	3 (10)	-
Chr2R	GPRgr13	26 (11)	26 (0)	0 (2)	NC
	GPRor39	36 (17)	36 (0)	0 (8)	NC
	GPRor38	33 (40)	33 (0)	0 (0)	NC
	FAC3C	20 (13)	16 (0)	4 (2)	-
Chr3R	Sterility	13 (9)	11 (0)	2 (4)	-
	tRNA syn	46 (38)	45 (0)	1 (19)	-
	GPRor69	35 (27)	32 (0)	3 (8)	-
	GPRor70	35 (23)	35 (0)	1 (9)	-
ChrX	P450-2	5 (8)	5 (5)	0 (0)	C
	Heat Shock	4 (3)	4 (1)	0 (0)	C
	P450-1	15 (9)	14 (0)	1 (4)	-
HEXAPODA: OTHER					
Abe *et al.* 2005	**Hempitera *Limniporus notabilis* x *L. dissortis***				
mtDNA	COI	64	64	0	RM
rDNA	ITS1	8	8	0	PA+M
autosomal	EF1A	27	27	0	RM
Bernasconi *et al.* 2010	**Hymenoptera *Formica paralugubris* x *F. lugubris***				
mtDNA	COI	2	2	0	RM
anonymous	Msat	65	-	-	C
Gangon & Turgeon 2009	**Hempitera *Gerris pingreensis* x *G. gillettei* x *G. incognitus***				
mtDNA	COI	18	16	2	-
autosomal	EF1A	8	6	2	-
Gomez-Zurita *et al.* 2006	**Coleoptera *Calligrapha abnicola* x *C. suturella***				
mtDNA	COI+COII+16S	19	19	0	RM
rDNA	ITS2	11	11	0	PO
autosomal	EF1A	16	16	0	PO
Jordal *et al.* 2006	**Coleoptera *Aphanarthrum subglabrum* x *A. glabrum***				
mtDNA	COI+16S	38	37	1	-
autosomal	enolase	58	58	0	RM

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
	EF1A	17	17	0	RM
	His3	3	2	1	-
Sota & Sasabe 2006 Coleoptera *Ohomopterus dehaanii* x *O. yaconius* x *O. iwawakianus* x *O. uenoi* x *O. yamato*					
mtDNA	ND5	18	14	4	-
autosomal	PepCK	72	70	2	-
	EF1A	86	86	0	PA/PO
Steiner *et al.* 2006 Hymenoptera *Myrmica microruba* x *M. ruba*					
mtDNA	COI+COII+Cytb	18	15	3	-
rDNA	ITS1	3	3	0	NC
anonymous	Msat	3	-	-	NC
Wirta 2009 Coleoptera *Nanos clypeatus* x *N. dubitatus* x *N. nitens* x *N. viettei*					
mtDNA	COI	64	64	0	PA
rDNA	ITS2	50	48	2	-
MISCELLANEOUS INVERTEBRATES					
Addison & Pogson 2009 Echinoidea *Strongylocentrotus droebachuensis* x *S. pallidus* x *S. purpuratus* x *S. franciscanus*					
mtDNA	nad5	36	35	1	-
autosomal	gp96	55	54	1	-
	cyc	65	63	2	-
	sm	66	63	3	-
sex-linked	soxB2	69	67	2	-
Chen & Hare 2008 Maxillopoda *Acartia tonsa "S"* x *"F"*					
mtDNA	COI	29	29	0	RM
autosomal	ITS	5	5	0	RM
DeWit & Erséus 2010 Clitellata *Grania pussila* x *G. variochaeta* x *G. postclitellochaeta* x *G. ovitheca*					
mtDNA	COI	87	87	0	RM
autosomal	ITS	39	39	0	RM
Frade *et al.* 2010 Anthozoa *Madracis pharensis* x *M. senaria* x *M. decactis* x *M. mirabilis* x *M. carmabi* x *M. formosa*					
mtDNA	nad5	5	3	2	-
autosomal	ATPS-A	33	31	2	-
	SRP54	61	54	7	-
Medina *et al.* 1999 Anthozoa *Montastraea annularis* x *M. faveolata* x *M. franksi*					
mtDNA	COI	2	1	1	-
autosomal	ITS	19	16	3	-
Naughton & O'Hara 2009 Asteroidea *Tosia neossia* x *T. magnifica* x *T. australis*					
mtDNA	COI+16S	36	36	0	RM
rDNA	ITS2	23	23	0	RM

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
Navajas & Boursot 2003 Arachnida *Tetronychus urticae* x *T. turkestoni*					
mtDNA	COI	11	11	0	PO
rDNA	ITS2	4	4	0	RM
Pérez-Losada *et al.* 2005 Clitellata *Eisenia andrei* x *E. fetida*					
mtDNA	COI	7	7	0	RM
rDNA	28S	9	9	0	RM
Puslednik *et al.* 2009 Gastropoda *Austropeplea tomentosa* x *A. lessoni*					
mtDNA	16S	42	42	0	PA+M
rDNA	ITS	42	42	0	PO+M
Reid *et al.* 2006 Gastropoda *Echinolittorina reticulata* x *E. millegrana*					
mtDNA	COI	46	46	0	PO
rDNA	28S	9	9	0	RM
Salomone *et al.* 2007 Arachnida *Euscorpius concinnus* x *E. sicanus*					
mtDNA	16S	21	21	0	RM
rDNA	ITS1	17	17	0	M+PA
Thum & Harrison 2009 Maxillopoda *Skistodiaptomus pallidus* x *S. pygmaeus* x *S. oregoensis*					
mtDNA	COI+cytb+16S	23	23	0	PO+M+M
rDNA	ITS1+2	14	14	0	RM
Yin *et al.* 2009 Malacostraca *Helice formosensis* x *H. latimera* x *H. tientsinensis*					
mtDNA	12S+16S+COI	39	38	1	-
rDNA	ITS2	5	2	3	-
FISHES					
Angienda *et al.* 2011 Perciformes *Oreochromis esculentus* x *O. noliticus*					
mtDNA	control-region	45	45	0	RM
anonymous	Msat	8	-	-	C
Houston *et al.* 2010 Cypriniformes *Richardsonius egregius* x *R. balteatus*					
mtDNA	cytb+CR	103	103	0	RM
rDNA	S7 intron	29	29	0	PO
Keck & Near 2010a Perciformes *Nothonotus microlepidus* x *N. sanguifluus*					
mtDNA	cytb	39	38	1	-
autosomal	MLL	6	3	3	-
	S7	42	4	1	-
	RAG-1	24	18	6	-
Keck & Near 2010b Perciformes *Nothonotus camurus* x *N. chlorobranchius* x *N. rufilineatus*					
mtDNA	cytb	189	180	9	-
rDNA	S7	173	173	0	PO

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
autosomal	MLL	57	55	2	-
	RAG-1	104	104	0	PA+M+M
López *et al.* 2010 Syngnathiformes *Hippocampus hippocampus* x *H. gulatas*					
mtDNA	16S	24	24	0	RM
anonymous	Msat	107	-	-	NC
Piggott *et al.* 2011 Characiformes *Cranegiella marthae "A", "B", "C"*					
mtDNA	ATPase 6 + 8	26	26	0	RM
anonymous	Msat	132	-	-	C
Rognon & Guyomard 2003 Perciformes *Oreochromis niloticus* x *O. aureus*					
mtDNA	cytb+cr	15	13	2	-
allozymes		14	-	-	C
Schultz *et al.* 2008 Carcharhiniformes *Negaprion brevirostris* x *N. acutidens*					
mtDNA	CR	15	15	0	RM
anonymous	Msat	9	-	-	C
Takahashi *et al.* 2007 Perciformes *Perissodus microlepis* x *P. straeleni* x *P. paradoxus* x *P. eccentricus* x *P. elaviae* x *P. multidentatus* x *P. hecqui*					
mtDNA	cytb	40	39	1	-
anonymous	AFLP	1582	-	-	C
Taylor & Hellberg 2006[f] Perciformes *Elacatinus chancei* x *E. horsti* x *E. lori* x *E. lousiae* [sponge-dwellers]					
mtDNA	cytb	23	23	0	PO+PO+RM+PO
autosomal	RAG-1	29	28	1	-
Taylor & Hellberg 2006[f] Perciformes *Elacatinus oceanops* x *E. evelynae* [cleaners]					
mtDNA	cytb	48	48	0	PO
autosomal	RAG-1	86	86	0	PO
AMPHIBIANS					
Bryson *et al.* 2010 Anura *Hyla arenicolor* x *H. wrightorum* x *H. eximia*					
mtDNA	ND4+ATPase	54	53	1	-
autosomal	POMC	30	30	0	M+PA
	cryB	22	21	1	-
Crawford 2003 Anura *Eleutherodactylus bransfordii* x *E. polyptychus* x *E. persimilis* x *E. stejnegerianus*					
mtDNA	ND2	21	21	0	RM
autosomal	cmy-c	38	38	0	PA/PO
Fitzpatrick *et al.* 2009 Anura *Thoropa miliaris* x *T. taophora*					
mtDNA	ND2+control region+16S	132	132	0	PA+M
autosomal	B-fibrinogen	28	28	0	RM
Fu & Zeng 2008 Caudata *Batrachuperus pinchonii* x *B. karlschmidti* x *B. taibaiensis* x *B. tibetanus* x *B. yenyuanensis* x *B. londongensis* x *B. "sp1"* x *B. "sp2"*					

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
mtDNA	cytB	116	116	0	RM
allozyme		145	-	-	C
Gamble *et al.* 2008 Anura *Acris blanchardi* x *A. creptians* x *A. gryllus*					
mtDNA	Cytb	61	61	0	RM
autosomal	tyrosinase	28	28	0	M+PA+M
	B-crystallin	23	23	0	PA+PA+M
	POMC	21	21	0	PA+PA+M
Gvoždík *et al.* 2010 Anura *Hyla savignyi* x *H. felixarabica*					
mtDNA	12S+16S	78	78	0	RM
autosomal	rhodopsin	5	5	0	RM
	tyrosinase	25	25	0	M+PA
Liu *et al.* 2010 Anura *Pelophylax plancyi* x *P. fukienensis* x *P. nigromaculata*					
mtDNA	Cytb	377	377	0	PO+M+PO
autosomal	TYR	76	76	0	M+PA+M
	POMC	137	137	0	RM
Lucas *et al.* 2009 Caudata *Eurycea pterophila* x *E. neotenes* x *E. nana*					
mtDNA	ND4	14	14	0	RM
autosomal	RAG-1	7	4	3	-
Matsui *et al.* 2008 Caudata *Salamandrella keyserlingii* x *S. schrenckii*					
mtDNA	cytb	39	39	0	RM
allozyme		16	-	-	RM
Timpe *et al.* 2009 Caudata *Eurycea aquatica* x *E. cirrigera*					
mtDNA	ND2	61	61	0	RM
autosomal	RAG-1	21	21	0	RM
Vogel & Johnson 2008 Anura *Bufo nebulifer* x *B. fowleri*					
mtDNA	COI	18	18	0	PA/PO
anonymous	SNP	4	0	4	-
Weisrock *et al.* 2006 Caudata *Ambystoma ordinarium* x *A. tigrinum complex*					
mtDNA	dloop	111	111	0	PO
autosomal	col1a1	50	50	0	M+PA
	ctg1908	27	27	0	M+PA
	g1c12	34	34	0	RM
	g1f1	26	26	0	PA
	g3d7	34	34	0	M+PA
	ctg1506	20	19	1	-
	g1d6	22	21	1	-
	dlx3	11	8	3	-
REPTILES					

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
Dolman & Moritz 2006 Squamata *Carlia rubrigularis* x *C. rhomboidalis*					
mtDNA	ND4	37	37	0	PA+M
autosomal	Adolase	18	17	1	-
	B-globin	30	30	0	PA+M
	GAPD	38	37	1	-
	MYH	71	71	0	PO
	Rhodopsin	35	32	3	-
	ets	17	12	5	-
anonymous	SK13/14	41	41	0	RM
Leaché *et al.* 2009 Squamata *Phrynosoma blainvillii* x *P. cerroense* x *P. coronatum*					
mtDNA	ND1+ND2+12S	87	87	0	PA+M+M
autosomal	RAG-1	36	32	4	-
	BDNF	14	11	3	-
Metzger *et al.* 2009 Squamata *Aspidomorphus muelleri* x *A. lineaticolis*					
mtDNA	cytb	42	42	0	RM
autosomal	SPTBN1	10	10	0	M+PA
Pinho *et al.* 2008 Squamata *Podarcis bocagei* x *P. carbonelli* x *P. vaucheri* x *P. hispanica "type IA"* x *"type IB"* x *"type 2"* x *P. hispanica s.s.* x *"type 3"* x *P. h. Galera* x *P. h. Jebel Sirwah* x *P. h. Tunisia* x *P. muralis*					
mtDNA	ND4	78	78	0	RM
autosomal	6-Pgint7	61	56	5	-
	B-fibint7	72	71	1	-
Rabosky *et al.* 2009 Squamata *Ctenotus leonhardii* x *C. quattuordecimlineatus*					
mtDNA	cytb	39	39	0	PA
autosomal	ATPSB	22	22	0	RM
	GAPDH	21	21	0	PA+M
BIRDS					
Bensch *et al.* 2006 Passeriformes *Phylloscopus trochilus* x *P. collybita*					
mtDNA	cytb	28	28	0	RM
autosomal	MCIR	34	33	1	-
W-chr	CHD-W	3	0	3	-
Z-chr	CHD-Z	16	16	0	PA+PO
anonymous	AFLP	24	-	-	C
Irwin *et al.* 2009 Passeriformes *Emberiza citrinella* x *E. leucocephalos*					
mtDNA	ND2	28	24	4	-
Z-chr	CHD1-Z	11	4	7	-
anonymous	AFLP	367	-	-	C
Kondo *et al.* 2008 Passeriformes *Icterus galbula* x *I. abeillei*					
mtDNA	cr+cytb	32	32	0	RM
autosomal	alpha-enolase	13	11	2	-
Z-chr	aldolaseB	4	3	1	-

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
Maley & Winker 2010 Passeriformes *Plectrophenax hyperboreus* x *P. nivalis*					
mtDNA	cytb	17	14	3	-
anonymous	AFLP	913	-	-	C
Pavlova *et al.* 2008 Passeriformes *Riparia riparia* x *R. diluta*					
mtDNA	ND2	59	59	0	RM
Z-chr	MUSK-13	8	8	0	RM
Peters *et al.* 2007 Anseriformes *Anas strepera* x *A. falcata*					
mtDNA	cr	57	56	1	-
autosomal	LDH	8	8	0	RM
Z-chr	CHD-Z	19	18	1	-
Welch *et al.* 2011 Procellariiformes *Pterodroma phaeopygia* x *P. sandwichensis*					
mtDNA	cytb	30	30	0	RM
autosomal	Enol	8	6	2	-
	Lam	21	20	1	-
	RP40	17	9	8	-
MAMMALS					
Berthier *et al.* 2006 Chiroptera *Myotis myotis* x *M. blythii*					
mtDNA	d-loop	58	53	5	-
anonymous	msats	5	-	-	C
Cabria *et al.* 2011 Carnivora *Mustela lutreola* x *M. putorius*					
mtDNA	control-region	29	12	17	-
ChrY	DDX3Y	2	0	2	-
anonymous	msat	74	-	-	C
Delton Hanson *et al.* 2010 Rodentia *Oryzomys palustris* x *O. couesi*					
mtDNA	cytb	94	94	0	RM
autosomal	Rbp3	29	29	0	RM
	Adhl-I2	29	29	0	RM
Evans *et al.* 2001 Primates *Macaca maura* x *M. tonkeana*					
mtDNA	12S	29	29	0	RM
ChrY	msat	1	-	-	C
anonymous	msats	9	-	-	C
Geraldes *et al.* 2008 Rodentia *Mus domesticus* x *M. musculus* x *M. castaneus*					
mtDNA	control-region	103	103	0	C+NC+NC
Chr1	Chrng	67	67	0	C+C+NC
Chr2	Med19	34	34	0	C+NC+NC
Chr3	Prpf3	47	47	0	NC+NC+C
Chr4	Clcn6	76	76	0	NC
ChrX	G6pdx	27	27	0	C+NC+NC

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
	Ocr1	50	50	0	C
ChrY	Jarid1d	81	81	0	C+NC+NC
Good *et al.* 2008 Rodentia *Tamias ruficaudus* x *T. amoenus*					
mtDNA	cytb	34	34	0	PO
autosomal	acr	10	10	0	RM
	acp5	12	12	0	RM
	c-myc	17	17	0	RM
	RAG1	15	14	1	-
anonymous	msats	7	-	-	C
Groeneveld *et al.* 2009 Primates *Cheirogaleus major* x *C. medius* x *C. crossleyi*					
mtDNA	cytb+COII	62	62	0	RM
autosomal	adora3	29	27	2	-
	fiba	49	49	0	RM
	VWF	52	52	0	RM
Hulva *et al.* 2010 Chiroptera *Pipistrellus pipistrellus* x *P. pygamaeus*					
mtDNA	D-loop	148	140	8	-
anonymous	msats	184	-	-	NC
Iwasa & Suzuki 2003 Rodentia *Eothenomys andersoni* x *E. smithii*					
mtDNA	cytb	35	35	0	PO+PA
rDNA	18S+ITS+28S	9	9	0	C
ChrY	Syr	6	6	0	C
Newbound *et al.* 2007 Chiroptera *Nyctimene cephalotes* x *N. albiventer*					
mtDNA	control-region	49	49	0	PA+M
allozymes		13	-	-	C
Pastorini *et al.* 2009 Primates *Eulemur fulvus* x *E. mongoz*					
mtDNA	ND4+D-loop	45	45	0	C
anonymous	msats	12	-	-	C
Rodriguez *et al.* 2010 Artiodactyla *Rupicapra rupicapra* x *R. pyrenaica*					
mtDNA	12S+ND1+Control-region +tRNApro	79	79	0	PO
anonymous	msats	20	-	-	NC
Yannic *et al.* 2010 Soricomorpha *Sorex granarius* x *S. araneus* x *S. antinorii* x *S. coronatus*					
autosomal	BRCA1	14	13	1	-
	ApoB	17	14	1	-
ChrX	AMELX	6	5	1	-
	ZFX1	10	9	1	-
anonymous	msats	7	-	-	NC
FUNGI					

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
Baayen *et al.* 2001 Hypocreales *Fusarium redolens* x *F. hostae*					
mtDNA	mtSSU	3	3	0	PA + M
autosomal	EF1A	7	7	0	RM
Barnes *et al.* 2004 Capnodiales *Dothistroma septosporum* x *D. pini*					
rDNA	ITS1-5.8S-ITS2	2	2	0	RM
autosomal	Btub1	6	6	0	RM
	EF1A	3	3	0	RM
Cortinas *et al.* 2006 Capnodiales *Colletogloeopsis zuluensis* x *C. gauchensis*					
mtDNA	ATP6	8	8	0	PA+M
rDNA	ITS1-5.8S-ITS2	6	6	0	RM
autosomal	Btub	8	8	0	RM
	EF1A	5	5	0	RM
Druzhinina *et al.* 2008 Hypocreales *Trichoderma longibranchiatum* x *Hypocrea orientalis*					
rDNA	ITS1-5.8S-ITS2	1	0	1	-
autosomal	EF1A	47	47	0	RM
	cal	40	40	0	M+PA
	chit18-5	38	38	0	RM
Groenewald *et al.* 2005 Capnodiales *Cercospora beticola* x *C. apii*					
rDNA	ITS1-5.8S-ITS2	1	0	1	-
autosomal	EF1A	2	1	1	-
	act	2	1	1	-
	cal	7	7	0	C
	his	2	1	1	-
anonymous	AFLP	-	-	-	C
Liu *et al.* 2009 Hypocreales *Moelleriella libera* x *M. raciborskii*					
autosomal	rpb2	21	21	0	PA+M
	EF1A	26	26	0	PA+M
	Btub	23	23	0	PA+M
Roe *et al.* 2010 Ophiostomatales *Grosmannia aurea* x *Leptographium terebrantis*					
rDNA	5.8S-ITS2-LSU	4	2	2	-
autosomal	Btub	7	7	0	RM
	EF1A	8	8	0	RM
	act	7	7	0	RM
	UFM	4	4	0	RM
Wulandari *et al.* 2009 Botryosphaeriales *Phyllosticta citriasiana* x *Guignardia citricarpa*					
rDNA	ITS1-5.8S-ITS2	2	2	0	C
autosomal	EF1A	6	6	0	C
	act	5	5	0	C
Yli-Mattila *et al.* 2004 Hypocreales *Fusarium langsethiae* x *F. sporotrichioides* x *F. poae*					

LOCATION	LOCI	TOTAL[a]	FIXED[b]	SHARED[c]	PHYLOGENETIC RECONSTRUCTION
rDNA	ITS1-5.8S-ITS2	6	5	1	-
	IGS	34	34	0	NC
autosomal	Btub	16	15	1	-

[a] TOTAL: Total number of haplotypes, or polymorphic sites (in parentheses), examined.

[b] FIXED: number of haplotypes, or polymorphisms (in parentheses), fixed for species limits.

[c] SHARED: Number of haplotypes, or polymorphisms (in parentheses), shared between species.

[d] Msat = Microsatellite locus

[e] Chr = Chromosome

[f] Study separated by ecological niche.

- = not applicable or unavailable.

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Appendix 2.6 All studies in literature survey, and results of sampling adequacy analysis, including number of specimens sampled per species (NO. SPEC./SPP.), the estimated extent of geographic sampling (SAMPLING EXTENT), and global distribution of each species (GEOG. DIST.). Sampling extent: 1 = ≤ 25%; 2 = ≤ 50%; 3 = ≤ 75%; and 4 = ≤ 100%. Global distribution: A = distribution is <100 km in diameter; B = distribution is <1000 km in diameter; C = distribution is between 1000 km and continent-wide (Americas, Eurasia, Africa, Australia/Australasia, and Antarctica), or for marine species, distribution is between 1000 and 5000 km in diameter; D = distribution spans more than one continent, or for marine species, distribution is > 5000 km in diameter. @ designates the minimum number of specimens sampled for all loci, in studies where different numbers of specimens were sampled for each locus.

Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
Abe *et al.* 2005	*Limnoporus notabilis*	9		1	C
	L. dissortis	9		1	C
Addison & Pogson 2009	*Strongylocentrotus droebachuensis*	20		1	D
	S. pallidus	20		1	D
	S. purpuratus	20		1	C
	S. franciscanus	10		1	C
Angienda *et al.* 2011	*Oreochromis esculentus*	60		3	B
	O. noliticus	120		1	C
Baayen *et al.* 2001	*Fusarium redolens*	29		NA	NA
	F. hostae	8		NA	NA
Bachtrog *et al.* 2006	*Drosophila yakuba*	41		2	C
	D. santomea	31		4	A
	D. teissieri	16		2	C
Barnes *et al.* 2004	*Dothistroma septosporum*	26		4	D
	D. pini	6		4	C
Bensch *et al.* 2006	*Phylloscopus trochilus*	33		4	C
	P. collybita	13		4	C
Bernasconi *et al.* 2010	*Formica paralugubris*	23	@	2	B
	F. lugubris	160	@	1	C
Berthier *et al.* 2006	*Myotis myotis*	7	@	3	C
	M. blythii	25	@	2	C
Besansky *et al.* 2003	*Anopheles gambiae*	12	@	3	C
	A. arabiensis	12	@	3	C
Bryson *et al.* 2010	*Hyla arenicolor*	61		4	C
	H. wrightorum	6		4	B
	H. eximia	5		4	B
Bull *et al.* 2006	*Heliconius cydno*	10		1	C
	H. melpomene	20		1	C

Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
Cabria *et al.* 2011	*Mustela lutreola*	313		2	C
	M. putorius	115		3	C
Chen & Hare 2008	*Acartia tonsa "S"*	36		1	D
	A. tonsa "F"	34		1	D
Cortinas *et al.* 2006	*Colletogloeopsis zuluensis*	30		NA	NA
	C. pini	23		NA	NA
Crawford 2003	*Eleutherodactylus bransfordii*	7		1	B
	E. polyptychus	10		1	B
	E. persimilis	9		1	B
	E. stejnegarius	29		2	B
Delton Hanson *et al.* 2010	*Oryzomys palustris*	46		3	C
	O. couesi	48		3	C
De Wit & Erséus 2010	*Grania pussila*	8		2	C
	G. variochaeta	10		3	C
	G. postclitellochaeta	8		2	C
	G. ovitheca	10		2	C
Dolman & Moritz 2006	*Carlia rubrigularis*	42		4	B
	C. rhomboidalis	14		3	B
Druzhinina *et al.* 2008*	*Trichoderma longibranchiatum*	37		NA	NA
	Hypocrea orientalis	10		NA	NA
Elias *et al.* 2007	*Mechanitis "mazaeus"*	5		1	C
	M. mazaeus	5		1	C
	M. lysimnia	6		1	C
	M. polymnia	7		1	C
Elias *et al.* 2007	*Hypothyris anastasia*	5		1	C
	H. euclea	5		1	C
	H. fluonia	5		1	C
	H. mamercus	7		1	C
	H. moebiusi	5		1	C
	H. semifulva	5		1	C
Elias *et al.* 2007	*Ithomia agnosia*	6		1	C
	I. amarilla	6		1	B
	I. salapia	5		1	C
Elias *et al.* 2007	*Melinaea marsaeus*	9		1	C
	M. menophilus	5		1	C
	M. satevis	6		1	C
Elkington *et al.* 2010	*Operophtera brumata*	28	@	1	D
	O. bruceata	37	@	2	C
Evans *et al.* 2001	*Macaca maura*	25		2	B
	M. tonkeana	27		4	B
Fitzpatrick *et al.* 2009	*Thoropa miliaris*	26		4	B
	T. taophora	129		4	B
Frade *et al.* 2010	*Madacris pharensis*	28		1	C
	M. senaria	20		2	C
	M. decactis	20		2	C
	M. mirabilis	26		2	C
	M. carmabi	13		1	C
	M. formosa	14		2	C
Fu & Zeng 2008	*Batrachuperus pinchonii*	112		4	B
	B. karlschmidtii	77		4	C
	B. taibaiensis	49		4	B

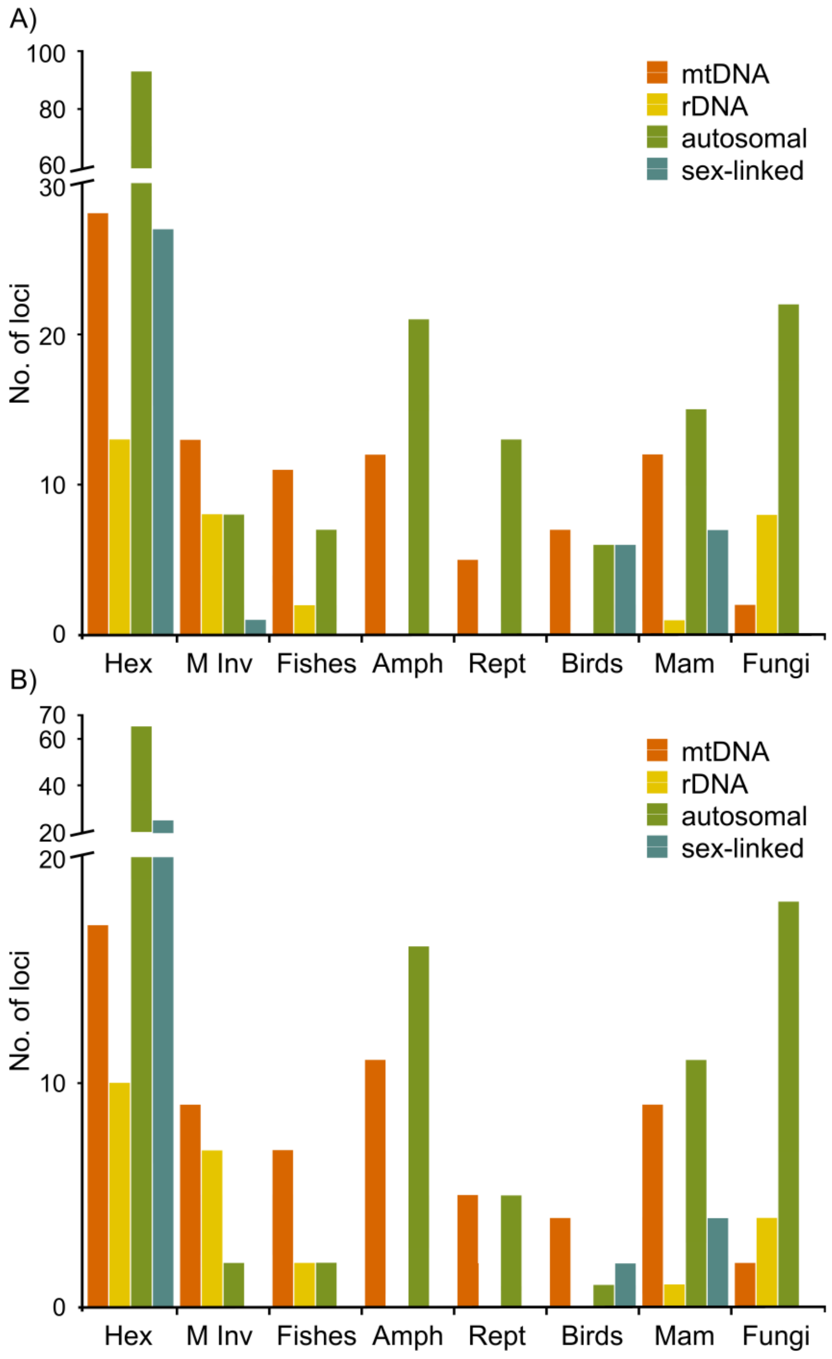
Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
	B. tibetanus	119		4	B
	B. yenyuanensis	12		4	B
	B. londongensis	51		4	B
	B. "sp1"	28		4	B
	B. "sp2"	12		4	B
Gamble *et al*. 2008	*Acris blanchardi*	32		4	C
	A. crepitans	11		2	C
	A. gryllus	21		3	C
Gangon & Turgeon 2010	*Gerris pingreensis*	28	@	4	C
	G. gilettei	11	@	4	C
	G. incognitus	16	@	4	C
Geraldes *et al.* 2008	*Mus domesticus*	60	@	3	C
	M. musculus	59	@	2	C
	M. castaneus	59	@	2	C
Gomez-Zurita *et al.* 2006	*Calligrapha abnicola*	12		3	C
	C. suturella	7		3	C
Gompert *et al.* 2006	*Lycaeides melissa*	81		1	C
	L. idas	77		1	C
	L. "alpine"	50		1	B
Good *et al.* 2008	*Tamias ruficaudus*	14	@	4	B
	T. amoenus	38	@	1	C
Groeneveld *et al.* 2009	*Cheirogaleus major*	31		4	C
	C. medius	28		4	C
	C. crossleyi	30		4	C
Groenewald *et al.* 2005	*Cercospora beticola*	14		NA	NA
	C. apii	11		NA	NA
Gvoždík *et al. 2010*	*Hyla savignyi*	26	@	4	C
	H. felixarabica	14	@	4	B
Hasan *et al.* 2008	*Anopheles farauti s.s.*	95	@	1	C
	A. irenicus	43	@	4	A
Hemmerter *et al*. 2009	*Culex annulirostris*	29		4	C
	C. a. "PNG"	12		4	B
	C. palpalis	17		4	C
Houston *et al.* 2010	*Richardsonius egregius*	7		4	B
	R. balteatus	22		4	C
Hulva *et al*. 2010	*Pipistrellus pipistrellus*	202		3	C
	P. pygamaeus	237		3	C
Irwin *et al*. 2009	*Emberiza citrinella*	156		3	C
	E. leucocephalos	87		2	C
Iwasa & Suzuki 2003	*Eothenomys andersoni*	9	@	4	B
	E. smithii	5	@	2	B
Jordal *et al*. 2006	*Aphanarthum subglabrum*	7	@	4	A
	A. glabrum	5	@	4	B
	A. nudum	5	@	4	A
Keck & Near 2010a	*Nothonotus microlepidus*	19		4	B
	N. sanguifluus	30		4	B
Keck & Near 2010b	*N. camurus*	52	@	3	B
	N. chlorobranchius	11	@	4	B
	N. rufilineatus	104	@	4	B
Kliman *et al.* 2000	*Drosophila simulans*	5	@	NA	NA
	D. sechelia	5	@	NA	NA

Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
	D. mauratiana	5	@	NA	NA
Kondo *et al*. 2008	*Icterus galbula*	26	@	3	C
	I. abeillei	22	@	1	B
Kronforst *et al*. 2006	*Heliconius cydno*	56		1	C
	H. melpomene	27		1	C
	H. pachinus	44		3	B
Leaché *et al*. 2009	*Phrynosoma blainvillii*	65		4	B
	P. cerroense	23		4	B
	P. coronatum	10		4	B
Liu *et al*. 2009	*Moelleriella libera*	20		NA	NA
	M. raciborskii	15		NA	NA
Liu *et al*. 2010	*Pelophylax plancyi*	320		4	C
	P. fukienensis	13		3	B
	P. nigromaculata	60		3	C
Llopart *et al*. 2005	*Drosophila yakuba*	11	@	1	C
	D. santomea	10	@	4	A
López *et al*. 2010	*Hippocampus hippocampus*	18		1	C
	H. gulatas	6		1	C
Lucas *et al*. 2009	*Eurycea pterophila*	95		1	B
	E. neotenes	56		1	B
	E. nana	103		1	B
Machado & Hey 2003	*Drosophila pseudoobscura*	19		NA	NA
	D. persimilis	14		NA	NA
	D. p. bogotana	11		NA	NA
Maley & Winker 2010	*Plectrophenax hyperboreus*	40		4	A
	P. nivalis	40		1	D
Matsui *et al*. 2008	*Salamandrella keyserlingii*	73		1	C
	S. schrenckii	23		NA	NA
Mazzoni *et al*. 2008	*Lutzomyia intermedia*	11	@	1	C
	L. whitmani	11	@	1	C
Medina *et al*. 1999	*Montastraea annularis*	9		1	C
	M. faveolata	7		1	C
	M. franksi	6		1	C
Metzeger *et al*. 2009	*Aspidomorphus muelleri*	15		2	C
	A. lineaticolis	19	@	4	B
Narita *et al*. 2006	*Eurema hecabe "Y type"*	39	@	4	C
	E. h. "B type"	14	@	4	C
Naughton & O'Hara 2009	*Tosia neossia*	9	@	4	C
	T. magnifica	8	@	4	C
	T. australis	13	@	4	C
Navajas & Boursot 2003	*Tetronychus urticae*	6	@	2	D
	T. turkestoni	6	@	3	D
Newbound *et al*. 2008	*Nyctimene cephalotes*	30	@	1	C
	N. albiventer	18	@	1	C
Pastorini *et al*. 2009	*Eulemur fulvus*	55		1	C
	E. mongoz	108		1	C
Pavlova *et al*. 2008	*Riparia riparia*	88	@	3	D
	R. diluta	33	@	2	C
Pérez-Losada *et al*. 2005	*Eisenia andrei*	20		1	D
	E. fetida	11		1	D
Peters *et al*. 2007	*Anas strepera*	42		4	D

Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
	A. falcata	47		3	C
Piggott *et al.* 2011	*Cranegiella marthae "A"*	60	@	2	C
	C. m. "B"	103		2	C
	C. m. "C"	241	@	2	C
Pinho *et al.* 2008	*Podacris bocagei*	5		4	B
	P. carbonelli	5		4	B
	P. vaucheri	5	@	2	C
	P. hispanica "type 1A"	6	@	3	B
	P. h. "type 1B"	5	@	4	B
	P. h. "type 2"	6	@	4	B
	P. hispanica s.s.	7		3	C
	P. h. "type 3"	5		3	B
	P. h. "Galera"	6		4	A
	P. h. "Jebel Sirwah"	6		4	A
	P. h. "Tunisia"	5		2	B
	P. muralis	5	@	1	D
Prudic *et al.* 2008	*Adelpha bredowii*	6	@	4	C
	A. eulalia	5	@	3	C
	A. californica	5	@	3	C
Puslednik *et al.* 2009	*Austropeplea tomentosa*	25		4	C
	A. lessoni	16		3	C
Rabosky *et al.* 2009	*Ctenotus leonhardii*	23		4	C
	C. quattuordecimlineatus	20		4	C
Reid *et al.* 2006	*Echinolittorina reticulata*	37		3	D
	E. millegrana	12		2	C
Rodriguez *et al.* 2010	*Rupicapra rupicapra*	49		3	C
	R. pyrenaica	26		4	B
Roe & Sperling 2007	*Dioryctria reniculelloides*	17	@	2	C
	D. pseudotsugella	12	@	3	C
Roe *et al.* 2010*	*Grosmannia aurea*	8		NA	NA
	Leptographium terebrantis	18		NA	NA
Rognon & Guyomard 2003	*Oreochromis niloticus*	28		1	C
	O. aureus	11		1	C
Rona *et al.* 2010	*Anopheles cruzii "Itaparica"*	21	@	1	C
	A. c. "Florianopolis"	15	@	1	C
Salomone *et al.* 2007	*Euscorpius concinnus*	22		1	C
	E. sicanus	20		1	C
Salvato *et al.* 2002	*Thaumetopoea pityocampa*	94		2	C
	T. wilkinsoni	10		1	C
Schoville *et al.* 2011	*Colias meadii*	6	@	1	C
	C. behrii	5	@	3	B
Schultz *et al.* 2008	*Negaprion brevirostris*	80		3	D
	N. acutidens	58		2	D
Sota & Sasabe 2006	*Ohomopterus dehaanii*	13		1	B
	O. yaconius	19	@	1	B
	O. iwawakianus	14	@	1	B
	O. uenoi	15		4	A
	O. yamato	10		1	B
Steiner *et al.* 2005	*Myrmica rubra*	116		3	C
	M. microrubra	107		3	C
Takahashi *et al.* 2007	*Perissodus microlepis*	66		2	B

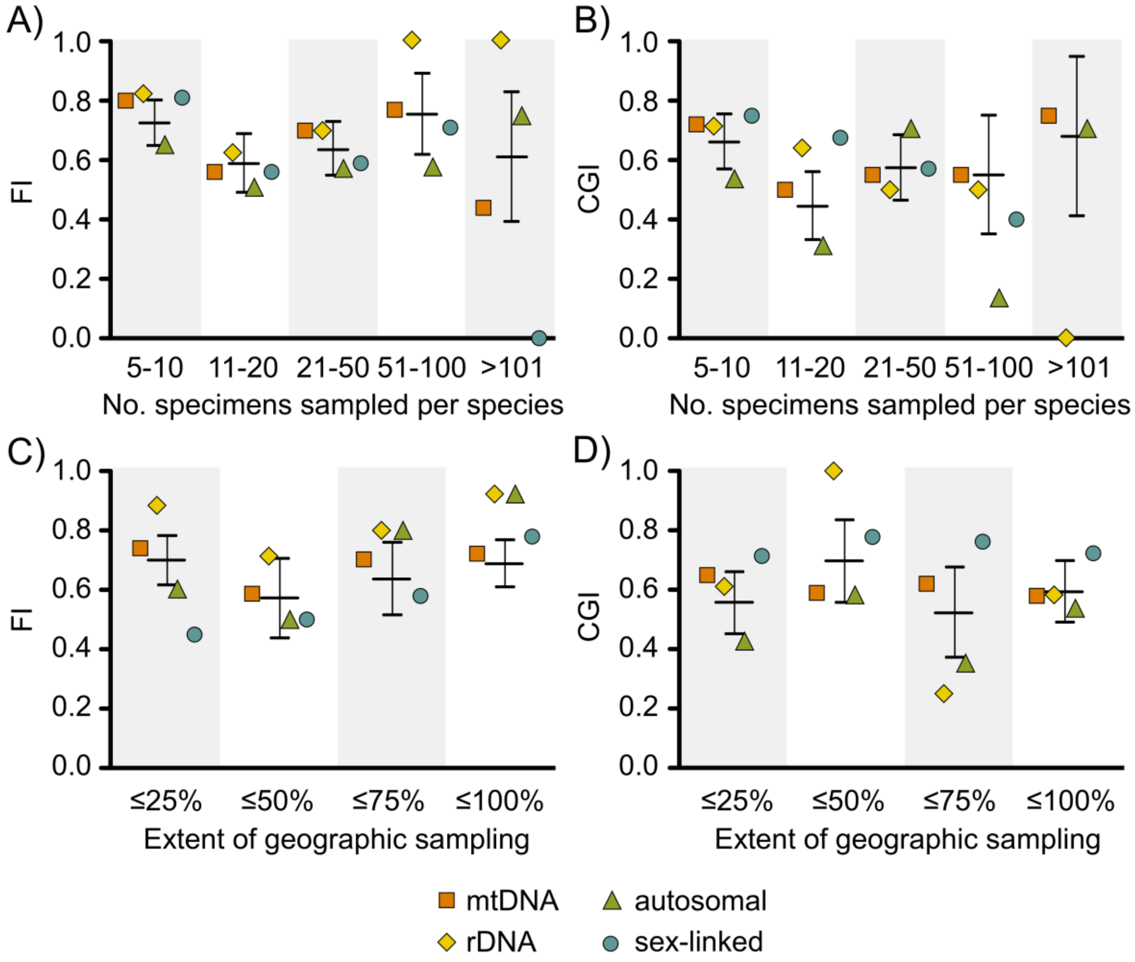
Study	Species	No. spec./spp.		Sampling Extent	Geog. Dist.
	P. straeleni	27		2	B
	P. paradoxus	26		2	B
	P. eccentricus	43		2	B
	P. elaviae	22		2	B
	P. multidentatus	35		2	B
	P. hecqui	36		2	B
Taylor & Hellberg 2006	*Elacatinus chancei*	24		1	C
	E. horsti	32		2	C
	E. lori	9		4	A
	E. lousiae	18		2	C
Taylor & Hellberg 2006	*E. oceanops*	20		1	C
	E. evelynae	80		4	D
Thum & Harrison 2009	*Skistodiaptomus pallidus*	9		2	C
	S. pygmaeus	7		1	C
	S. oregoensis	13		2	C
Timpe *et al.* 2009	*Eurycea aquatica*	17	@	3	B
	E. cirrigera	10	@	1	C
Turner *et al.* 2005	*Anopheles gambiae "M form"*	7		1	C
	A. g. "S form"	7		1	C
Vogel & Johnson 2008	*Bufo nebulifer*	40		1	C
	B. fowleri	31		1	C
Weisrock *et al.* 2006	*Ambystoma ordinarium*	NA		4	A
	A. tigrinum	NA		4	C
Welch *et al.* 2011	*Pterodroma phaeopygia*	35		2	B
	P. sandwichensis	28		2	B
Wirta 2009	*Nanos clypeatus*	12	@	2	A
	N. dubitatus	38	@	3	B
	N. nitens	10		1	A
	N. viettei	29		4	B
Wulandari *et al.* 2009*	*Phyllosticta citriasiana*	11		NA	NA
	Guignardia citricarpa	8		NA	NA
Yannic *et al.* 2010	*Sorex granarius*	9		2	B
	S. araneus	15		1	C
	S. antinorii	12		3	B
	S. coronatus	11		1	C
Yin *et al.* 2009	*Helice formosensis*	9	@	NA	NA
	H. latimera	11	@	NA	NA
	H. tientsinensis	12	@	NA	NA
Yli-Mattila *et al.* 2004	*Fusarium langsethiae*	22		NA	NA
	F. sporotrichioides	33		NA	NA
	F. poae	42		NA	NA

*Fungal species that belong to different genera, but are considered in the same clade.

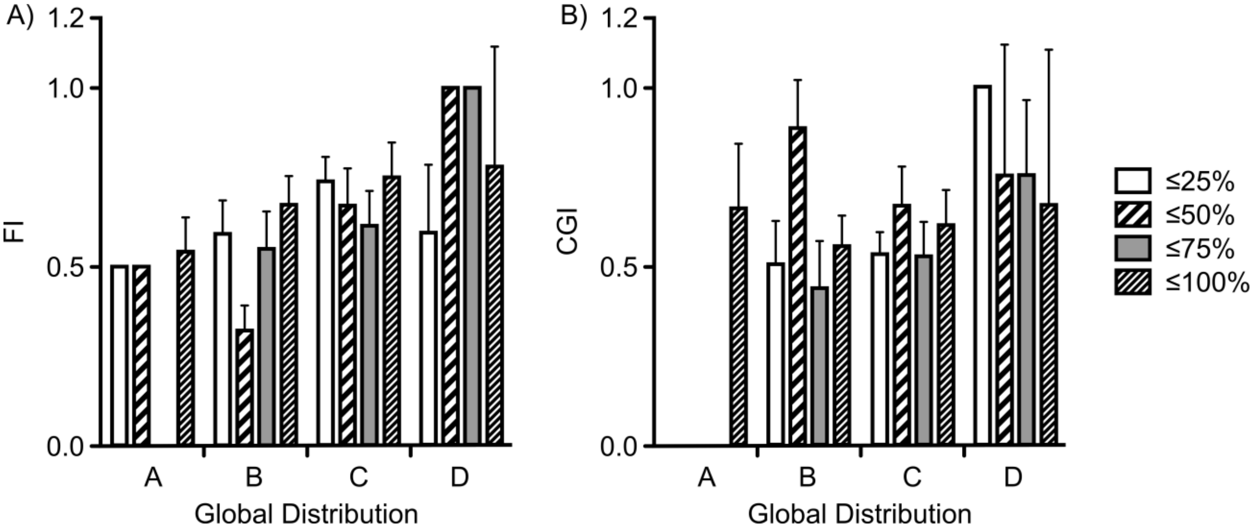


Appendix 2.7 Number of separately used loci for each marker category and organism group showing: A) fixed differences among species versus shared haplotypes, and B) haplotype clades/clusters that are congruent versus non-congruent with species. Column height corresponds to number of loci assessed in our literature survey, with empty spaces indicating marker

categories that were absent for that organism group. The solid-colored lower portion of the column corresponds to the number of loci with fixed haplotype differences (A) or congruent haplotypes (B). The faded upper portion of the column represents the number of loci with shared or non-congruent haplotypes. Hex: hexapods; M Inv: miscellaneous invertebrates; Amph: amphibians; Rept: reptiles; Mam: mammals.



Appendix 2.8 Sampling adequacy divided by marker type: A) fixation, and B) congruence indices categorized by the number of specimens sampled per species, and C) fixation, and D) congruence indices categorized by the extent of geographic sampling. Black lines with error bars represent the mean fixation/clustering and 95% confidence intervals of each category without regard to marker type. Marker types do not have equal sample sizes and so overall FI/CGI means are not necessarily centered among the different marker values.



Appendix 2.9 A) Fixation and B) congruence indices divided by both extent of geographic sampling and estimated global distribution. Only studies where global distributions were estimated are included (n=246 species). Columns represent average fixation or clustering values for each category with 95% confidence intervals. Columns with no confidence intervals are represented by only one species. Some combinations of global distribution and extent of sampling have no data (and hence no column). Global distribution: A = distribution is <100 km in diameter; B = distribution is <1000 km in diameter; C = distribution is between 1000 km and continent-wide (Americas, Eurasia, Africa, Australia/Australasia, and Antarctica), or for marine species, distribution is between 1000 and 5000 km in diameter; D = distribution spans more than one continent, or for marine species, distribution is > 5000 km in diameter.

Appendix 3.1 Specimen information. COI/COII and EF-1α columns provide GenBank accession numbers followed by the haplotype names used in the present study; for COI/COII, haplotype names in parentheses are those used in Sperling & Harrison (1994) (Some specimens were used in Sperling & Harrison (1994), but were not sequenced for this study). Italicized GenBank accession numbers represent sequences not generated in this study. Asterisks in columns S and M indicate specimens used for microsatellite and morphometric analyses, respectively.

#	Species	Locality; collector; year collected; host (if available); latitude longitude (if available)	COI/COII	EF-1α	S	M
FS223.h15	*P. machaon aliaska*	USA: Alaska: Eagle Summit; Ayres, M.P.; 1990; 65.48453 -145.40346	*FJ808909.1* mach3			
FS260	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Troubridge, J.T.; 1990; 57.05197 -122.86839	KJ363206 mach2(M11)	KJ363312 mach2	*	*
JRD136	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363246 mach19		*	*
JRD139	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363247 mach19		*	*
JRD150	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363249 mach19		*	*
JRD152	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363248 mach19		*	*
JRD156	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363250 hyb12		*	*
JRD158	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363253 zel10		*	*
JRD164	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KJ363257 zel10		*	*
JRD169	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Dupuis, J.R.; 2010; 57.05197 -122.86839	KP262869 hyb12		*	*
JRD415	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Sperling, F.A.H.; 1998; 57.05197 -122.86839	KP262873 mach19		*	*

JRD669	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Schmidt, B.C.; 2005; 57.05197 -122.86839	KP262874 hyb12		*	*
JRD670	*P. machaon aliaska*	Canada: British Columbia: Alaska Hwy: Pink Mtn; Schmidt, B.C.; 2005; 57.05197 -122.86839	KP262875 hyb12		*	*
FS176	*P. machaon bairdii*	USA: California: San Bernardino co.: Sugarloaf Mtn.; Emmel, J.F.; 1990; 34.19918 -116.81460	KJ363223 mach4(M3)			*
FS188	*P. machaon bairdii*	USA: California: San Bernardino co.: Sugarloaf Mtn.; Emmel, J.F.; 1990; 34.19918 -116.81460	KJ363224 mach4(M3)			*
FS194	*P. machaon bairdii*	USA: Arizona: Apache co.: Eagar; McCorkle, D.V.; 1989	KJ363225 mach19(M4)			*
FS211	*P. machaon bairdii*	USA: California: San Bernardino co.: Sugarloaf Mtn.; Emmel, J.F.; 1990; 34.19918 -116.81460	KJ363231 mach19(M3)		*	*
FS204	*P. machaon bairdii (brucei)*	USA: Nebraska: west Nebraska: Dawson co.; Spomer, S. via Heitzman, J.R.; 1990	KJ363203 mach1(M5)	KJ363309 mach1	*	*
FS265	*P. machaon bairdii(brucei)*	USA: Nebraska: west Nebraska; Spomer, S. via Heitzman, J.R.; 1990	KJ363234 mach19(M2)		*	*
FS277	*P. machaon bairdii(brucei)*	USA: Colorado: Freemont co.; Fisher, M.S.; 1990	KJ363235 mach19(M3)		*	*
FS155	*P. machaon dodi*	USA: Montana: Circle; Sperling, F.A.H.; 1987	KJ363219 mach6(M2)		*	*
JRD209	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363258 mach5		*	*
JRD210	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363259 mach5		*	*
JRD211	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363260 mach5		*	
JRD223	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363261 mach5		*	*
JRD224	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363262 mach5		*	*
JRD227	*P. machaon dodi*	Canada: Alberta: Drumheller: Tolman Bridge; Dupuis, J.R.; 2010; 51.84258 -113.00796	KJ363263 mach5		*	*
FS027	*P. machaon gorganus*	France: Coudoux; Piquemal L., via Hauser, C.; 1987	*AF044006.1* mach15(M1)	AF044819.1 mach15	*	*
FS156	*P. machaon gorganus*	Czech Republic: Prague; Häuser, C.; 1987	KJ363220 mach16(M3)		*	*
FS078	*P. machaon hippocrates*	Japan: Gifu Pref.: Vicinity of Gifu; Okura, J.; 1989	*AY457593.1* mach14(M8)	*AY457621.1* mach14	*	*

FS323	*P. machaon hippocrates*	Japan: Tokyo Prefecture: Mount Takao; West, D.A.; 1990	KJ363241 mach17(M8)			*
FS431	*P. machaon hippocrates*	Japan: Aichi Prefecture: Nagoya; Ae, S.A.; 1990	(M8)		*	*
JRDB073	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262876 hyb12		*	*
JRDB074	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262877 mach8		*	*
JRDB076	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262878 hyb12		*	*
JRDB077	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262879 hyb12		*	*
JRDB078	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262880 hyb12		*	*
JRDB079	*P. machaon hudsonianus*	Canada: Manitoba: N of Duck Mtn.; Dupuis, J.R.; 2014; 51.9944 -101.0353	KP262881 hyb12		*	*
JRDB098	*P. machaon hudsonianus*	Canada: Québec: E of Chisasibi: salt marsh; Larrivee, M.; 2010; on *Ligusticum scoticum*	KP262882 hyb12			
FS077	*P. machaon oregonius*	USA: Washington: Palouse Falls; Anderson, S.S.; 1988; 46.656509 -118.228575	*AF044007.1* mach9(M3)	*AF044828.2* mach9		*
FS234	*P. machaon oregonius*	USA: Washington: Klickitat co.; McCorkle, D.V.; 1990	(M3)		*	*
FS377	*P. machaon oregonius*	USA: Washington: Wishram: on Columbia River; McCorkle, D.V.; 1986	(M3)		*	*
2300.h2	*P. machaon pikei*	Canada: Alberta: Peace River: S of Bear Canyon; Baker, A. & Szkoropa, T.; 2002; 56.16706 -119.81085	*FJ808896.1* mach13			
2804.h5	*P. machaon pikei*	Canada: Alberta: S of Peace River: Rd. to Judah; Bromilow S., Schmidt C.; 2005; 56.151 -117.315	*FJ808899.1* hyb13			
FS263	*P. machaon pikei*	Canada: British Columbia: Taylor; Troubridge, J.T.; 1990; 56.15403 -120.71828	KJ363207 mach12(M10)	KJ363313 mach12	*	*
JRD175	*P. machaon pikei*	Canada: Alberta: Peace River: Highland Park N; Dupuis, J.R.; 2010; 56.13076 -118.88931	KJ363251 mach11		*	*
JRD177	*P. machaon pikei*	Canada: Alberta: Peace River: Highland Park N; Dupuis, J.R.; 2010; 56.13076 -118.88931	KJ363252 mach11		*	*
JRD180	*P. machaon pikei*	Canada: Alberta: Peace River: Kaufman Hill; Dupuis, J.R.; 2010; 56.24883 -117.27408	KJ363254 mach19		*	*

JRD181	*P. machaon pikei*	Canada: Alberta: Peace River: Kaufman Hill; Dupuis, J.R.; 2010; 56.24883 -117.27408	KJ363255 mach19		*	*
JRD182	*P. machaon pikei*	Canada: Alberta: Peace River: Kaufman Hill; Dupuis, J.R.; 2010; 56.24883 -117.27408	KJ363256 mach11		*	*
FS082b	*P. polyxenes americus*	Ecuador: Quito; Ponce, P. via Emmel, J.F.; 1989	KJ363196 poly1(P2)	KJ363302 poly1	*	*
FS102	*P. polyxenes americus*	Ecuador: Quito; Ponce, P. via Emmel, J.F.; 1989	KJ363216 poly9(P2)		*	*
FS110	*P. polyxenes americus*	Ecuador: Napo Prov.: Baños; Maudsley, J.R.; 1989	(P1)			*
FS274	*P. polyxenes americus*	Ecuador: Ambato; Levasseur, J. via McCorkle, D.V.; 1990	(P3)		*	*
FS013	*P. polyxenes asterius*	USA: New York: Watkins Glen; Sperling, F.A.H.; 1987; on *Pastinaca sativa*; 42.379 -76.871	KJ363211 poly2(P1)		*	*
FS064	*P. polyxenes asterius*	USA: New York: Ithaca: Feeny culture; Feeny, P.; 1989	*AF044010.1* poly3(P1)	*AF044823.2* poly3	*	*
FS087	*P. polyxenes asterius*	USA: Pennsylvannia: Pine Grove; Houtz, W.; 1989	KJ363215 poly9(P1)		*	
FS199	*P. polyxenes asterius*	USA: Missouri: Benton co.: Truman State Park; Heitzman, J.R.; 1990; 38.2687 -93.4463	KJ363227 poly9()		*	*
FS206	*P. polyxenes asterius*	USA: Michigan: Mackinac co.; Herig, T.; 1990	KJ363229 poly9(P1)		*	*
FS207	*P. polyxenes asterius*	Canada: Ontario: Port Hope: Rod Parrott culture; Parrot, R.; 1990	(P1)		*	*
FS221	*P. polyxenes asterius*	USA: Missouri: Lees Summit; Heitzman, J.R.; 1990; on parsley; 38.8988 -94.3832	(P1)		*	*
FS317	*P. polyxenes asterius*	USA: Missouri: Independence; Heitzman, J.R.; 1990; on *Foeniculum vulgare*; 39.1144 -94.4391	(P1)		*	*
JRDB003	*P. polyxenes asterius*	USA: South Carolina: ; Zakharov, E.; 2002	KJ363296 poly9		*	*
JRDB004	*P. polyxenes asterius*	USA: North Carolina: Stanleyville: Ziglar Rd.; Sperling, F.A.H.; 2000; 36.19497 -80.28906	KJ363297 poly4		*	*
JRDB006	*P. polyxenes asterius*	USA: Pennsylvania: N of Ligonier: Wineland Rd.; Hilchie, G.J.; 2012; 40.2905 -79.2019	KJ363270 poly9		*	
FS080	*P. polyxenes coloro*	USA: California: Hemet; Emmel, J.F.; 1989; on *Ruta graveolens*	(P1)			*
FS177	*P. polyxenes coloro*	USA: California: San Diego co.: Jacumba; Griffin, B. via Emmel, J.F.; 1989; 32.61617 -116.18943	(P1)		*	*
FS191	*P. polyxenes coloro*	USA: Arizona: Maricopa co.: Four Peaks Rd.; Griffin, B. via Emmel, J.F.; 1989; 33.66964 -111.49151	KJ363201 poly5(P1)	KJ363307 poly5	*	*

FS218	*P. polyxenes coloro*	USA: California: Riverside co.: Hemet; Emmel, J.F.; 1990; on *Ruta graveolens*; 33.7203 -116.9343	KJ363232 poly9(P1)		*	*
JRDB009	*P. polyxenes coloro*	USA: Arizona: Portal: AMNH SW Research Station; Anweiler, G.; 2012; 31.912676 -109.141157	KJ363298 poly6		*	*
JRDB010	*P. polyxenes coloro*	USA: Arizona: Portal: AMNH SW Research Station; Anweiler, G.; 2012; 31.912676 -109.141157	KJ363299 poly7		*	*
JRDB012	*P. polyxenes coloro*	USA: Arizona: Portal: AMNH SW Research Station; Anweiler, G.; 2012; 31.912676 -109.141157	KJ363271 poly9		*	
FS220	*P. polyxenes stabilis*	Costa Rica: Monteverde; Joyce, F.; 1990; on *Anethum graveolens*	KJ363204 poly8(P1)	KJ363310 poly8	*	*
FS051	*P. zelicaon*	USA: California: Hemet; Emmel, J.F.; 1989; 33.7203 -116.9343	KJ363213 zel10(Z1)		*	*
FS052	*P. zelicaon*	USA: Washington: Juniper Dunes; Wehling, W.; 1989; 46.38883 -118.85631	KJ363214 zel10(Z1)		*	*
FS060	*P. zelicaon*	USA: South Dakota: Black Hills: near Silver City; Sperling, F.A.H.; 1987; 44.07693 -103.5725	KJ363195 zel4(Z5)	KJ363301 zel4		
FS076	*P. zelicaon*	USA: California: Riverside co.: Hemet; Emmel, J.F.; 1989; 33.7203 -116.9343	*AF044008.1* zel5(Z1)	*AF044827.1* zel5	*	*
FS153	*P. zelicaon*	USA: South Dakota: Black Hills: near Silver City; Sperling, F.A.H.; 1987; on *Zizia aptera*; 44.07693 -103.5725	KJ363218 zel10(Z4)		*	*
FS161	*P. zelicaon*	USA: South Dakota: Black Hills: near Silver City; Sperling, F.A.H.; 1987; 44.07693 -103.5725	KJ363222 zel2(Z6)		*	*
FS165	*P. zelicaon*	USA: California: Hemet; Emmel, J.F.; 1990; on *Foeniculum vulgare*; 33.7203 -116.9343	KJ363273 zel10()		*	*
FS174	*P. zelicaon*	USA: South Dakota: Black Hills: near Silver City; Sperling, F.A.H.; 1987; 44.07693 -103.5725	KJ363274 zel10(Z4)		*	*
FS182	*P. zelicaon*	USA: Colorado: Jefferson co.: Lookout Mtn.; Fisher, M.S.; 1990; 39.7336 -105.2380	KJ363199 zel1(Z6)	KJ363305 zel1_1	*	
FS183	*P. zelicaon*	USA: Colorado: Jefferson co.: Lookout Mtn.; Fisher, M.S.; 1990; 39.7336 -105.2380	KJ363275 zel2(Z6)		*	*
FS186	*P. zelicaon*	USA: Washington: Colockum Pass Rd.; Peterson, M.A.; 1990; 47.2608 -120.1873	KJ363276 zel10(Z1)		*	*

FS187	*P. zelicaon*	USA: Washington: Wilson Creek Rd.; Peterson, M.A.; 1990; 47.0874 -120.4981	KJ363200 zel1(Z4)	KJ363306 zel1_2	*	*
FS208	*P. zelicaon*	USA: Colorado: Jefferson co.: Lookout Mtn.; Fisher, M.S.; 1990; 39.7336 -105.2380	KJ363230 zel6(Z4)		*	*
FS229	*P. zelicaon*	USA: South Dakota: Black Hills: near Silver City; Sperling, F.A.H.; 1987; 44.07693 -103.5725	KJ363233 zel10(Z4)		*	*
FS284	*P. zelicaon*	USA: Washington: Columbia River: Rock Ck.; McCorkle, D.V.; 1990	KJ363237 zel3(Z1)		*	*
FS296	*P. zelicaon*	USA: Colorado: Jefferson co.: Lookout Mtn.; Fisher, M.S.; 1990; 39.7336 -105.2380	KJ363239 zel2(Z6)		*	*
FS393	*P. zelicaon*	Canada: British Columbia: Vancouver area; Troubridge, J.T.; 1990; 49.2 -122.8	KJ363243 zel2(Z4)		*	
FS394	*P. zelicaon*	Canada: British Columbia: Vancouver area; Troubridge, J.T.; 1990; 49.2 -122.8	KJ363244 zel3(Z1)		*	
FS420	*P. zelicaon*	USA: California: Hemet; Emmel, J.F.; 1989; 33.7203 -116.9343	KJ363245 zel2(Z4)		*	
JRD295	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills E; Dupuis, J.R.; 2011; 51.25993 -112.45478	KJ363264 zel2		*	*
JRD296	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills E; Dupuis, J.R.; 2011; 51.25993 -112.45478	KJ363265 zel2		*	*
JRD301	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills W; Dupuis, J.R.; 2011; 51.25520 -112.62614	KJ363266 zel10		*	
JRD302	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills W; Dupuis, J.R.; 2011; 51.25520 -112.62614	KJ363267 zel2		*	*
JRD303	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills E; Dupuis, J.R.; 2011; 51.25993 -112.45478	KJ363268 zel2		*	*
JRD306	*P. zelicaon*	Canada: Alberta: Drumheller: Wintering Hills E; Dupuis, J.R.; 2011; 51.25993 -112.45478	KJ363269 zel2		*	*
FS319	*P. brevicauda brevicauda*	Canada: Newfoundland: Fishell; Tremblay, N.; 1990	KJ363193 hyb1(M12)		*	*
FS320	*P. brevicauda brevicauda*	Canada: Newfoundland: Fishell; Tremblay, N.; 1990	KJ363240 hyb12(M12)		*	*
JRDB001	*P. brevicauda brevicauda*	Canada: Newfoundland: LaScie; Anweiler, G.; 2012; 49.9577 -55.6119	KJ363294 hyb2		*	*
JRDB002	*P. brevicauda brevicauda*	Canada: Newfoundland: LaScie; Anweiler, G.; 2012; 49.9577 -55.6119	KJ363295 hyb12		*	*

FS056	*P. brevicauda gaspeensis*	Canada: Quebec: Gaspe; Sperling, F.A.H.; 1988; on *Heracleum lanatum*	KJ363194 hyb11(M12)	KJ363300 hyb11_2		*
FS281	*P. brevicauda gaspeensis*	Canada: New Brunswick: Shippegan; McCloud, E. via Berenbaum, M.; 1990; on *Logusticum scoticum*	KJ363236 hyb12(M12)		*	*
FS321	*P. brevicauda gaspeensis*	Canada: Quebec: Gaspe; Tremblay, N.; 1990	KJ363210 hyb11(M12)	KJ363316 hyb11_3	*	*
FS120	*P. joanae*	USA: Missouri; Yoon, C. via McCorkle, D.V.; 1990	KJ363217 hyb12()		*	*
FS160	*P. joanae*	USA: Missouri; Yoon, C. via McCorkle, D.V.; 1990	KJ363221 hyb12()		*	*
FS198	*P. joanae*	USA: Missouri: Benton co.: 2.5 mi N of Truman State Park; Heitzman, J.R.; 1990; 38.31256 -93.44157	KJ363226 hyb12()		*	*
FS227	*P. joanae*	USA: Missouri: Benton co.: 2.5 mi N of Truman State Park; Heitzman, J.R.; 1990; 38.31256 -93.44157	KJ363205 hyb3(M12)	KJ363311 hyb3	*	*
FS288	*P. joanae*	USA: Missouri: Benton co.: 2.5 mi N of Truman State Park; Heitzman, J.R.; 1990; 38.31256 -93.44157	(M12)		*	*
FS311	*P. joanae*	USA: Missouri: C. Yoon culture from R. Heitzmann; Heitzman, J.R. via McCorkle, D.V.; 1986	KJ363209 hyb11(M13)	KJ363315 hyb11_1	*	*
FS312	*P. joanae*	USA: Missouri: Benton co.: W of Warsaw; Heitzman, J.R.; 1990; 38.24686 -93.40773	KJ363192 hyb4(M12)		*	*
FS279	*P. machaon kahli*	Canada: Manitoba: near Winnipeg; McCorkle, D.V.; 1990; 50.0014 -96.9167	()		*	
FS290	*P. machaon kahli*	Canada: Manitoba: Winnipeg: Bird Hill Park; McCorkle, D.V. via Hansen, K.; 1987; 50.0014 -96.9167	KJ363238 hyb5(M12)		*	*
FS328	*P. machaon kahli*	Canada: Manitoba: near Winnipeg; McCorkle, D.V.; 1990; 50.0014 -96.9167	KJ363242 hyb6()		*	*
JRDB015	*P. machaon kahli*	Canada: Manitoba: near Winnipeg; McCorkle, D.; 2005; 50.0014 -96.9167	KJ363272 hyb7		*	*
FS034	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H.; 1987; on *Zizia aptera*; 50.91694 -114.53558	KJ363212 hyb9(M12)		*	*
FS151	*P. zelicaon x machaon*	Canada: Alberta: Buck Mtn.; Sperling, F.A.H.; 1987; on *Heracleum lanatum*; 53.05209 -114.73961	KJ363197 zel8(Z3)	KJ363303 zel8	*	*

FS201	*P. zelicaon x machaon*	Canada: Alberta: Buck Mtn.; Kondla, N.G.; 1990; 53.05209 -114.73961	KJ363228 zel9(Z3)		*	*
JRD103	*P. zelicaon* x *machaon*	Canada: Alberta: Buck Mtn.; Sperling, F.A.H.; 2010; 53.05209 -114.73961	KJ363277 zel7		*	*
JRD195	*P. zelicaon* x *machaon*	Canada: Alberta: Nordegg: Shunda Lkt.; Dupuis, J.R., Brunet, B.; 2010; 52.48259 -115.73825	KP262870 zel7		*	*
JRD197	*P. zelicaon* x *machaon*	Canada: Alberta: Nordegg: Shunda Lkt.; Dupuis, J.R., Brunet, B.; 2010; 52.48259 -115.73825	KP262871 zel10		*	*
JRD198	*P. zelicaon* x *machaon*	Canada: Alberta: Nordegg: Shunda Mtn.; Dupuis, J.R., Brunet, B.; 2010; 52.53151 -116.12610	KJ363278 zel10		*	*
JRD202	*P. zelicaon* x *machaon*	Canada: Alberta: Nordegg: Shunda Mtn.; Dupuis, J.R., Brunet, B.; 2010; 52.53151 -116.12610	KJ363279 zel10		*	*
JRD321	*P. zelicaon* x *machaon*	Canada: Alberta: Buck Mtn.; Dupuis, J.R.; 2011; 53.05209 -114.73961	KJ363280 zel7		*	*
JRD322	*P. zelicaon* x *machaon*	Canada: Alberta: Buck Mtn.; Dupuis, J.R.; 2011; 53.05209 -114.73961	KJ363281 zel10		*	*
JRD389	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H., Sperling, E., Sperling, T.; 2011; 50.91694 -114.53558	KJ363283 hyb8		*	*
JRD391	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H., Sperling, E., Sperling, T.; 2011; 50.91694 -114.53558	KJ363284 hyb8		*	*
JRD392	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H., Sperling, E., Sperling, T.; 2011; 50.91694 -114.53558	KP262872 zel11		*	*
JRD393	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H., Sperling, E., Sperling, T.; 2011; 50.91694 -114.53558	KJ363285 mach18		*	
JRD395	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck. Ski Hill; Dupuis, J.R.; 2011; 50.98175 -114.58286	KJ363286 zel10		*	*
JRD396	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck. Ski Hill; Dupuis, J.R.; 2011; 50.98175 -114.58286	KJ363287 mach18		*	*
JRD401	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Mesa Butte; Dupuis, J.R.; 2011; 50.78071 -114.56134	KJ363288 mach18		*	

JRD640	*P. zelicaon* x *machaon*	Canada: Alberta: Kananaskis Country: Powderface Mtn.; Dupuis, J.R.; 2012; 50.84316 -114.84863	KJ363289 zel10		*	*
JRD641	*P. zelicaon* x *machaon*	Canada: Alberta: Kananaskis Country: Powderface Mtn.; Dupuis, J.R.; 2012; 50.84316 -114.84863	KJ363290 hyb8		*	*
JRD645	*P. zelicaon* x *machaon*	Canada: Alberta: Kananaskis Country: Powderface Mtn.; Dupuis, J.R.; 2012; 50.84316 -114.84863	KJ363291 mach18		*	
JRD681	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H.; 2001; 50.91694 -114.53558	KJ363292 hyb10		*	*
JRD686	*P. zelicaon* x *machaon*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H.; 2001; 50.91694 -114.53558	KJ363293 hyb12		*	*
JRD386	*P. zelicaon* x *machaon (nitra)*	Canada: Alberta: Bragg Ck.: Fish Butte; Sperling, F.A.H., Sperling, E., Sperling, T.; 2011; 50.91694 -114.53558	KJ363282 mach18		*	*
FS143	*P. hospiton*	Sardinia; Crnjar, R. via Clarke, C.A.; 1989	*AF044009.1* hosp(H)	*AF044830.1* hosp		*
FS066	*P. indra indra*	USA: Washington: Wawawai co.: Wehling/Thompson culture; Wehling, W.; 1987	*AF044011.1* indra2(I)	*AF044824.1* indra1_1	*	*
FS181	*P. indra indra*	USA: Colorado: Jefferson co.: Lookout Mtn.; Fisher, M.S.; 1990; 39.7336 -105.2380	KJ363198 indra1(I)	KJ363304 indra1_3	*	*
FS267	*P. indra indra*	USA: Washington: Kittitas co.: N Fork Teanaway Riv; Peterson, M.A.; 1990; 47.2556 -120.8807	KJ363208 indra1(I)	KJ363314 indra1_2	*	*
FS197	*P. indra kaibabensis*	USA: Arizona: Coconino co.:13.2 mi W of Cameron; Griffin, B. via Emmel, J.F.; 1990; on *Lomatium parryi*; 35.8765 -111.6339	KJ363202 indra1(I)	KJ363308 indra2	*	*
FS238	*P. xuthus*	Japan: Tokyo Prefecture: Mount Takao; Taguchi, M. & Sperling, F.A.H.; 1990; 35.62496 139.24378	*AF043999.1* xuthus(X)	*AF044838.2* xuthus		

Appendix 3.2 Microsatellite loci used in this study (from Zakharov & Hellman 2007). A “T” following the primer name indicates loci that were PIG-tailed (see methods). Abbreviations: T_a: annealing temperature, N: number of alleles observed, Range: allelic size range observed.

Locus	T_a (°C)	N	Range
PZ-A229T	57	15	203-239
PZ-B209T	56	11	188-208
PZ-B225T	57	23	210-300
PZ-B102T	57	24	173-247
PZ-A121	57	24	259-327
PZ-B12T	57	24	245-327
PZ-A110	57	17	217-255
PZ-A117T	57	32	249-357
PZ-A214	56	27	191-301
PZ-D224*	57	28	171-288

*primer sequences for PZ-D224 were misreported in Zakharov & Hellman (2007). Correct primer sequences are F(5’-3’): CACCATCATCAACAACAACC and R: TTGGTAGTGTTCCTTGACCAC.

Appendix 3.3 Primers for new sequences used in this study. * corresponds to antisense or minority direction primers. Alternate names for primers are given in parenthesis. Reference positions are relative to *Drosophila yakuba* (Clary & Wolstenholme 1985) for COI, and *Heliothodes diminutivus* (Cho *et al.* 1995) for EF-1α.

Gene	Name	Reference Position (3' end)	Sequence (5' → 3')
COI	Jerry	2183	CAA CAT TTA TTT TGA TTT TTT GG
	Mila*	2659	GCT AAT CCA GTG AAT AAT GG
	k741*	2578	TGG AAA TGT GCA ACT ACA TAA TA
	Pat*	3014	TCC AAT GCA CTA ATC TGC CAT ATT A
EF-1α	Bo (M44-1)	225	GCT CG(CT) GA(AG) CGT GGT ATC AC
	Juke (E600rc)*	620	CTC CTT ACG CTC AAC ATT CC
	Verdi3*	795	GAC ACC AGT TTC AAC TCT GCC

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Appendix 3.4 (Next pages, after references) Summary of morphological and ecological information pertinent to the species included in the present study. Information was abstracted from the sources below:

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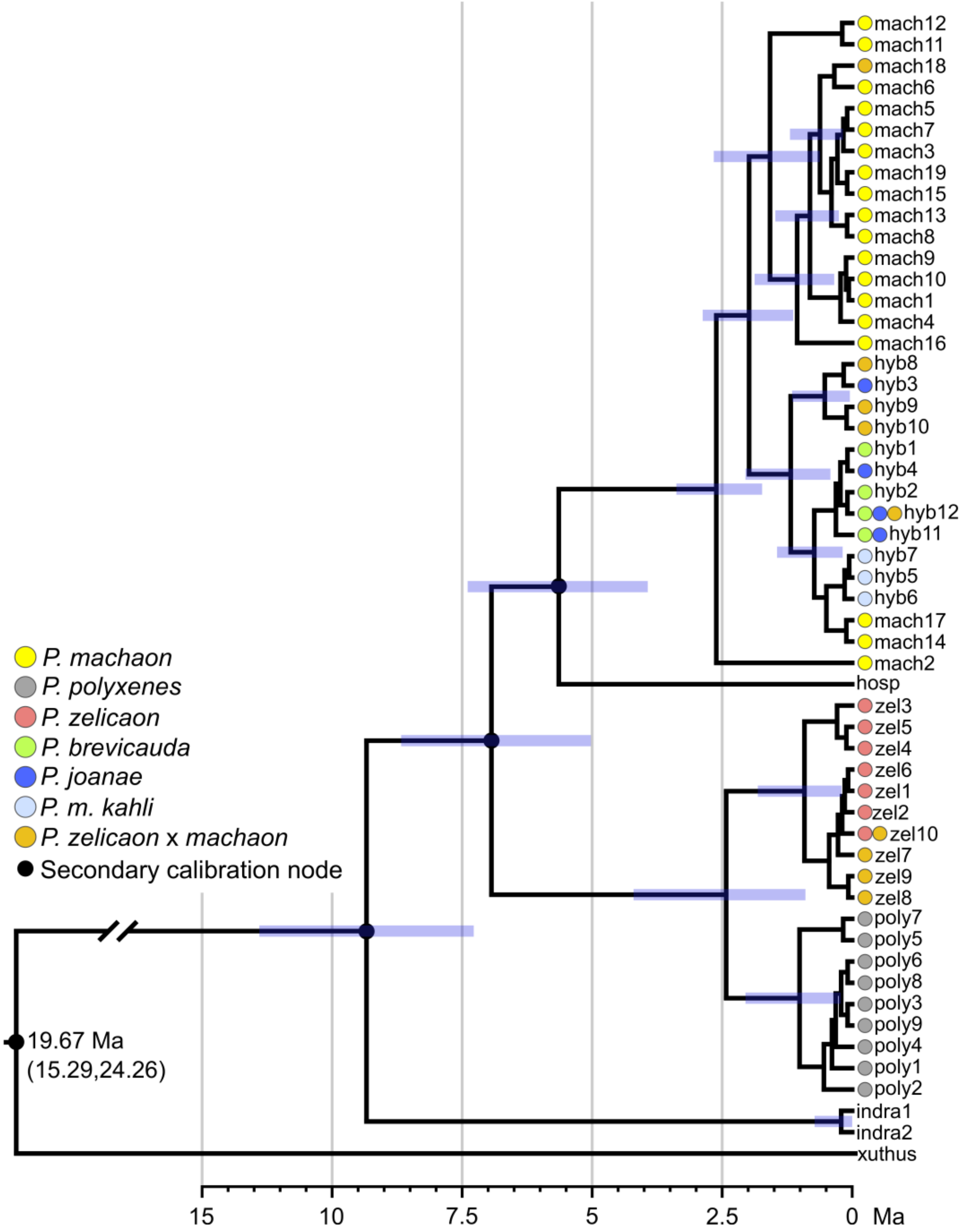
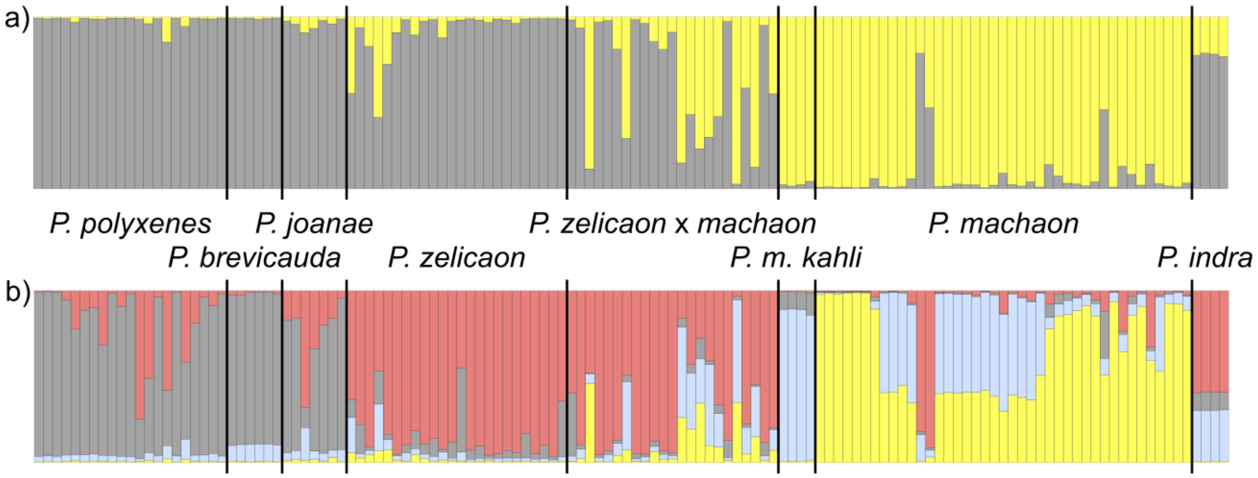
Species	Subspecies	Distinctive wing characters	Flight period	Habitat	Host
P. machaon	Many subspecies across Eurasia, northern and western North America	Generally yellow background, pointed wing tips, long tails, eyespot connected to wing margin	Variable and habitat dependent	Generally strict hilltoppers	Asteraceae in arid or northern habitats, Apiaceae in mesic habitats
	aliaska	Flatter eyespot pupil, shorter tails and more rounded wing tips than other North American *P. machaon*	June-July	Alpine tundra and mountain tops	*Artemesia arctica, Cnidium cnidiifolium, Petasites frigidus*
	pikei	Similar to *P. m. oregonius*	June-July	Dry grassland and eroding banks	*Artemesia dracunculus*
	hudsonianus	Similar to *P. m. aliaska*, more club-shaped eyespot	June-July	Hilltops and boreal forest openings	*Heracleum lanatum* (?)*, Ligusticum scothicum, Petasites palmatus/frigidus, Zizia aptera* (?)
	dodi/bairdii/brucei/oregonius	Mostly yellow morph, but black morphs common in some regions	Multiple flights, variable; Alberta: May-August	Dry grassland and tops of high, eroding river banks	*Artemesia dracunculus*
P. polyxenes	Two subspecies in North America, others extending into South America	Generally black background (more yellow in American Southwest and South America), pointed wing tips, long tails, eyespot central, not connected to wing margin	Range dependent and variable	Variable, many hilltop, but also frequent open garden habitats in eastern North America	Wide variety of Apiaceae and occasional Rutaceae, often locally restricted

Species	Subspecies	Distinctive wing characters	Flight period	Habitat	Host
(P. polyxenes)	*asterius*	As above	Manitoba: late May-late June; North-east: late May-mid June and mid July to late September	As above	*Anethum graveolens, Angelica spp., Apium graveolens, Berula erecta, Carum carvi, Cicuta spp., Conium maculatum, Cryptotaenia canadensis, Cymopterus panamintensis, Daucus spp., Dictamnus albus, Foeniculum vulgare, Harboria trachypleura, Heracleum spp., Levisticum officinale, Ligusticum scothicum, Osmorhiza longistylis, Oxypolis canbyi, Pastinaca sativa, Petroselinum crispum, Ptilimnium capillaceum, Ruta graveolens, Sium suave, Spermolepis divaricata, Taenidia integerrima, Tauschia spp., Thamnosma spp., Thaspium barbinode, Zizia spp.*
	coloro	Often more yellow scaling ranging to yellow morph individuals	March-September (often rain dependent)	As above	*Cymopterus panamintensis, Daucus carota, Foeniculum vulgare, Lomatium parryi, Petroselinum crispum, Ruta graveolens, Tauschia spp., Thamnosma montana*
	americus/stabilis	Yellow background common	Many flights, close to all year	As above	*Apium leptophyllum, Spananthe paniculata, Cyclospermum leptophyllum*
P. zelicaon	Subspecies not currently recognized, geographic variation exists	Generally yellow background (regionally black), rounder wing tips, eyespot central, not connected to wing margin	Alberta: May-late July; south: March-September	Generally strict hilltoppers	*Anethum graveolens, Angelica spp., Apium graveolens, Carum carvi, Cicuta caulata, Citrus spp., Conioselinum scopulorum, Conium maculatum, Cymopterus spp., Daucus spp., Foeniculum vulgare, Harbouria trachypleura, Heracleum spp.* (incl. *lanatum*), *Ligusticum spp., Lomatium spp., Musineon tenuifolium, Oenanthe sarmentosa, Pastinaca sativa, Perideridia spp., Petroselinum crispum, Pimpinella spp., Pseudocymopterus montanus, Pteryxia spp., Ruta spp., Sium suave, Sphenosciadium capitellatum, Tauschia spp., Zizia aptera*

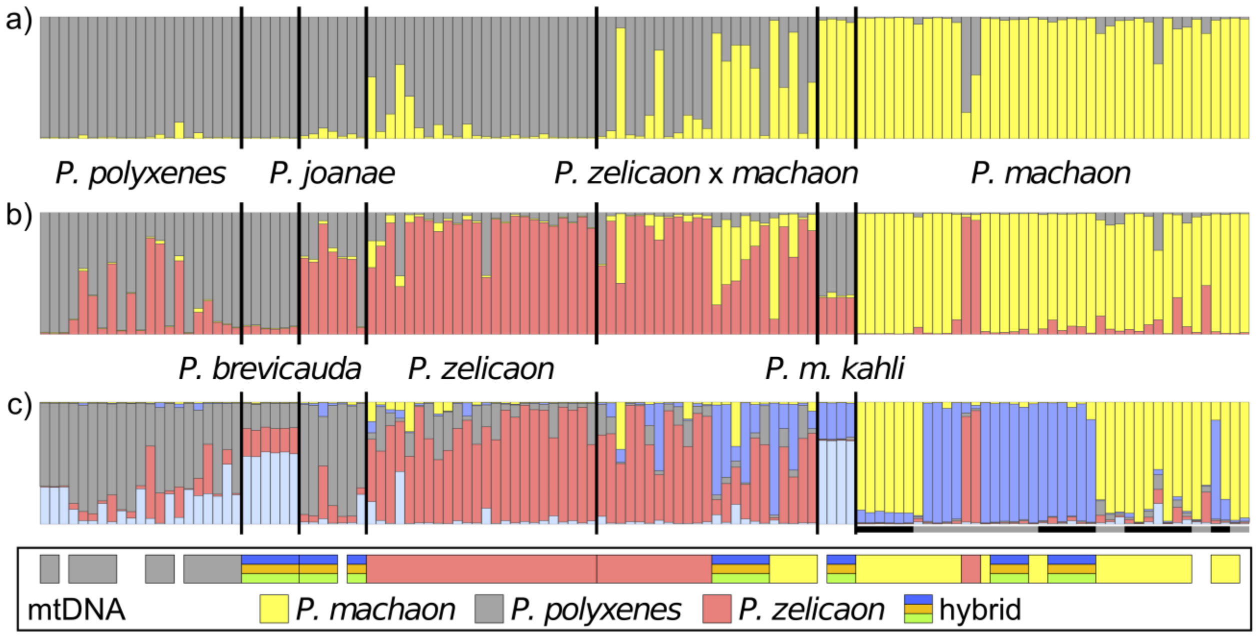
Species	Subspecies	Distinctive wing characters	Flight period	Habitat	Host
P. brevicauda	Three subspecies currently recognized, ecological characteristics do not differ between subspecies	Black background, rounded wing tips, shorter tails than most *P. machaon*	Mid June-late July	Hilltopping behavior common, particularly on ocean bluffs; also frequent lowland marsh and open garden habitats	*Angelica atropurpurea, Apium graveolens, Coelopleurum lucidum, Conioselinum chinense, Daucus carota, Heracleum lanatum, Ligusticum scothicum, Pastinaca sativa, Petroselinum crispum*
P. joanae	No subspecies recognized	Often indistinguishable from *P. polyxenes*, sometimes darker, with eyespot variably connected to wing margin	Multiple flights, May-September	Strictly flies under forest cover	*Taenidia integerrima, Thaspium barbinode, Zizia aurea*
P. m. kahli	*kahli* currently recognized as subspecies of *P. machaon*	Generally black background, pointed wing tips, long tails, eyespot connected to wing margin	Late May-late June	Variable, as in *P. polyxenes*	*Heracleum spp., Pastinaca sativa, Petroselinum crispum, Zizia spp.*
P. zelicaon x *machaon*		Generally yellow background (although black morphs relatively common), shorter, rounded wing tips, slightly shorter tails, eyespot extremely variable	May-July	Hilltopping behavior as in *P. zelicaon*	*Heracleum lanatum, Lomatium spp., Zizia aptera*



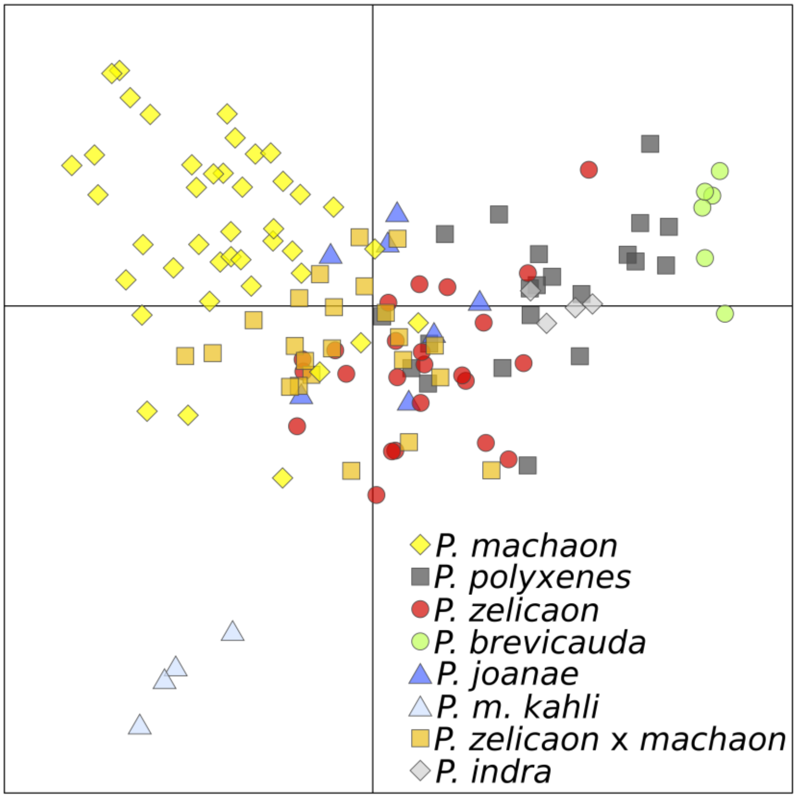
Appendix 3.5 Molecular dated tree based on COI/COII data secondarily calibrated with four nodes. 95% confidence intervals shown with blue bars.



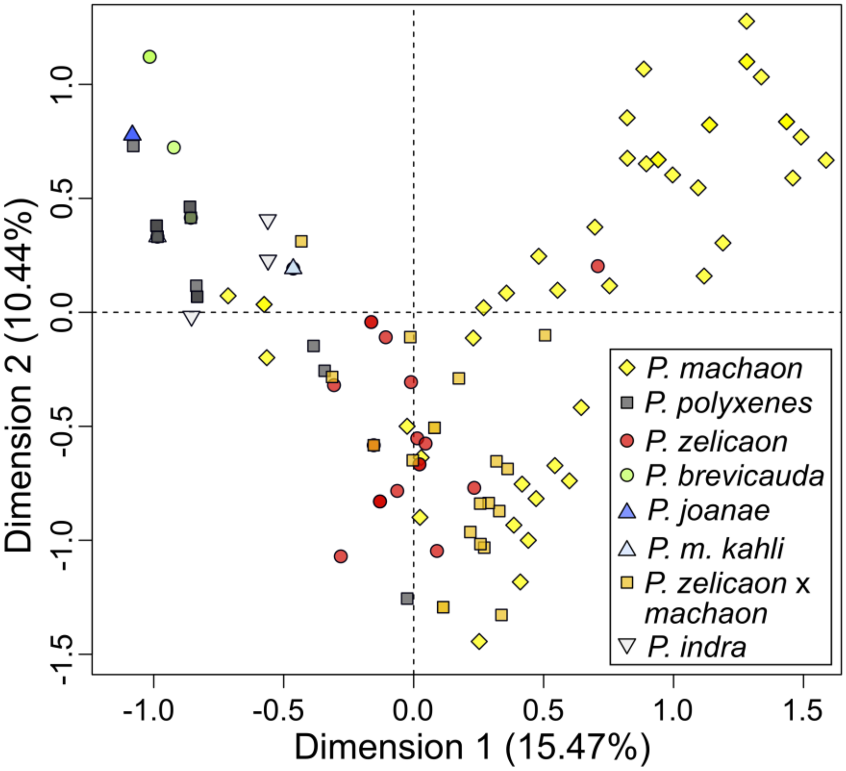
Appendix 3.6 STRUCTURE results for all microsatellite data, including *P. indra.* a) $k = 2$, and b) $k = 4$.



Appendix 3.7 STRUCTURE results for microsatellite genotype data for the overall dataset, including substructure. a) $k = 2$ (identical to Figure 3), b) $k = 3$, and c) $k = 5$. Inset indicates the major mtDNA clade (*P. machaon*, *P. polyxenes*, *P. zelicaon*, or the main hybrid clade within the *P. machaon* clade) for each individual (gaps indicate specimens genotyped for microsatellites that were not sequenced for COI/COII). Alternating black and grey bars below the *P. machaon* portion of c) indicate subspecies; from left to right: *P. m. pikei*, *aliaska*, *hudsonianus*, *bairdii*, *dodi*, *oregonius*, *gorganus*, and *hippocrates*.



Appendix 3.8 DAPC for all microsatellite data, including *P.indra*.



Appendix 3.9 Morphometric MCA without using the *jitter* function to separate overlapping points.

Appendix 4.1 Individual collection data. Asterisk in locality information indicates that specimen was collected as a caterpillar. mtDNA refers to main mtDNA clade (M: *P. machaon*, Z: *P. zelicaon*, H: hybrid).

Ind #s	species	region	locality (latitude, longitude) collection date; collector	mtDNA
JRD315 UASM270315	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 30.v.2011; Dupuis, JR	Z
JRD540 UASM270540	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD541 UASM270541	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD542 UASM270542	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD543 UASM270543	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD544 UASM270544	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD545 UASM270545	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD546 UASM270546	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD547 UASM270547	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD548 UASM270548	*Papilio zelicaon*	RDR	AB: Hand Hills West (51.53452, -112.3661) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD539 UASM270539	*Papilio zelicaon*	RDR	AB: Little Fish Lake (51.39252, -112.21149) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD580 UASM270580	*Papilio zelicaon*	RDR	AB: Little Fish Lake (51.39252, -112.21149) 1.vi.2012; Dupuis, JR	Z

JRD536 UASM270536	*Papilio zelicaon*	RDR	AB: S of East Coulee mine (51.3162, -112.48393) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD537 UASM270537	*Papilio zelicaon*	RDR	AB: Thumb Hill (51.35877, -112.28907) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD538 UASM270538	*Papilio zelicaon*	RDR	AB: Thumb Hill (51.35877, -112.28907) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD295 UASM270295	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 17.v.2011; Dupuis, JR	Z
JRD296 UASM270296	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 17.v.2011; Dupuis, JR	Z
JRD297 UASM270297	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 18.v.2011; Dupuis, JR	Z
JRD298 UASM270298	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 18.v.2011; Dupuis, JR	Z
JRD300 UASM270300	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 18.v.2011; Dupuis, JR	Z
JRD303 UASM270303	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	Z
JRD304 UASM270304	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	Z
JRD306 UASM270306	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	Z
JRD307 UASM270307	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	Z
JRD309 UASM270309	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 29.v.2011; Dupuis, JR	Z
JRD317 UASM270317	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 30.v.2011; Dupuis, JR	Z
JRD318 UASM270318	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 30.v.2011; Dupuis, JR	Z
JRD564 UASM270564	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD565 UASM270565	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD566 UASM270566	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD568 UASM270568	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD569 UASM270569	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD571 UASM270571	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD573 UASM270573	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z

JRD574 UASM270574	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD576 UASM270576	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD577 UASM270577	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD578 UASM270578	*Papilio zelicaon*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	Z
JRD301 UASM270301	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 18.v.2011; Dupuis, JR	Z
JRD302 UASM270302	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 18.v.2011; Dupuis, JR	Z
JRD313 UASM270313	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 29.v.2011; Dupuis, JR	Z
JRD314 UASM270314	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 29.v.2011; Dupuis, JR	Z
JRD319 UASM270319	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 30.v.2011; Dupuis, JR	Z
JRD320 UASM270320	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 30.v.2011; Dupuis, JR	Z
JRD524 UASM270524	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD525 UASM270525	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD526 UASM270526	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD527 UASM270527	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD528 UASM270528	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD529 UASM270529	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD530 UASM270530	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD531 UASM270531	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD532 UASM270532	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z

JRD533 UASM270533	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD534 UASM270534	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	Z
JRD557 UASM270557	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD558 UASM270558	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD559 UASM270559	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD560 UASM270560	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD561 UASM270561	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD562 UASM270562	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD563 UASM270563	*Papilio zelicaon*	RDR	AB: Wintering Hills West (51.2552, -112.62614) 1.vi.2012; Dupuis, JR	Z
JRD249 UASM270249	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD254 UASM270254	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD255 UASM270255	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD256 UASM270256	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD257 UASM270257	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD259 UASM270259	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD263 UASM270263	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD267 UASM270267	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD269 UASM270269	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD275 UASM270275	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD276 UASM270276	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD278 UASM270278	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD490 UASM270490	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M

JRD494 UASM270494	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD496 UASM270496	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD501 UASM270501	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD502 UASM270502	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD503 UASM270503	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD504 UASM270504	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD505 UASM270505	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD512 UASM270512	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD515 UASM270515	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD646 UASM270646	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD651 UASM270651	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2010; Dupuis, JR	M
JRD652 UASM270652	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 11.viii.2012; Dupuis, JR	M
JRD727 UASM270727	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD730 UASM270730	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD733 UASM270733	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD736 UASM270736	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD738 UASM270738	*Papilio machaon dodi*	RDR	AB: Horsethief Canyon* (51.50378, -112.92857) 23.viii.2011; Dupuis, JR	M
JRD264 UASM270264	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD277 UASM270277	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD281 UASM270281	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD287 UASM270287	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD288 UASM270288	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD292 UASM270292	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M

JRD293 UASM270293	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 19.viii.2010; Dupuis, JR	M
JRD487 UASM270487	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD497 UASM270497	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD499 UASM270499	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD500 UASM270500	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD508 UASM270508	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD509 UASM270509	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD510 UASM270510	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD511 UASM270511	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD513 UASM270513	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD516 UASM270516	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD518 UASM270518	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD520 UASM270520	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD521 UASM270521	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD734 UASM270734	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD739 UASM270739	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD740 UASM270740	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD743 UASM270743	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD744 UASM270744	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD745 UASM270745	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD747 UASM270747	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD748 UASM270748	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD750 UASM270750	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M

JRD751 UASM270751	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD752 UASM270752	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD754 UASM270754	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD848 UASM270848	*Papilio machaon dodi*	RDR	AB: Lousana* (52.07838, -113.00907) 22.viii.2011; Dupuis, JR	M
JRD245 UASM270245	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD246 UASM270246	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD247 UASM270247	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD248 UASM270248	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD250 UASM270250	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD251 UASM270251	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD252 UASM270252	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD253 UASM270253	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD258 UASM270258	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD261 UASM270261	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD262 UASM270262	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD266 UASM270266	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD272 UASM270272	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD273 UASM270273	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD274 UASM270274	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD280 UASM270280	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD289 UASM270289	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD522 UASM270522	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 23.viii.2011; Dupuis, JR	M
JRD649 UASM270649	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M

JRD650 UASM270650	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 12.viii.2010; Dupuis, JR	M
JRD737 UASM270737	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 23.viii.2011; Dupuis, JR	M
JRD742 UASM270742	*Papilio machaon dodi*	RDR	AB: Morrin Bridge* (51.64462, -112.92085) 23.viii.2011; Dupuis, JR	M
JRD001 UASM270001	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 14.v.2010; Dupuis, JR	M
JRD002 UASM270002	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 14.v.2010; Dupuis, JR	M
JRD019 UASM270019	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 26.v.2010; Dupuis, JR	M
JRD020 UASM270020	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 26.v.2010; Dupuis, JR	M
JRD021 UASM270021	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 26.v.2010; Dupuis, JR	M
JRD222 UASM270222	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD223 UASM270223	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD224 UASM270224	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD225 UASM270225	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD226 UASM270226	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD227 UASM270227	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD228 UASM270228	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 21.vii.2010; Dupuis, JR	M
JRD229 UASM270229	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD230 UASM270230	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M

JRD231 UASM270231	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD232 UASM270232	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD233 UASM270233	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD234 UASM270234	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD235 UASM270235	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD236 UASM270236	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 17.vii.2010; Sperling, FAH, Fagua, G	M
JRD311 UASM270311	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 29.v.2011; Dupuis, JR	M
JRD312 UASM270312	*Papilio machaon dodi*	RDR	AB: N of Drumheller Town (51.47276, -112.70495) 29.v.2011; Dupuis, JR	M
JRD260 UASM270260	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD265 UASM270265	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD270 UASM270270	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD271 UASM270271	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD279 UASM270279	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD282 UASM270282	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD283 UASM270283	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD284 UASM270284	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD290 UASM270290	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD291 UASM270291	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD514 UASM270514	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 23.viii.2011; Dupuis, JR	M
JRD519	*Papilio machaon*	RDR	AB: Orkney Lkt* (51.54887, -	M

UASM270519	*dodi*		112.89692) 23.viii.2011; Dupuis, JR	
JRD647 UASM270647	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD648 UASM270648	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 12.viii.2010; Dupuis, JR	M
JRD746 UASM270746	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 23.viii.2011; Dupuis, JR	M
JRD749 UASM270749	*Papilio machaon dodi*	RDR	AB: Orkney Lkt* (51.54887, -112.89692) 23.viii.2011; Dupuis, JR	M
JRD535 UASM270535	*Papilio machaon dodi*	RDR	AB: S of East Coulee mine (51.3162, -112.48393) 20.v.2012; Dupuis, JR, Mori, B, Higuera, M	M
JRD579 UASM270579	*Papilio machaon dodi*	RDR	AB: S of East Coulee mine (51.3162, -112.48393) 1.vi.2012; Dupuis, JR	M
JRD209 UASM270209	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD210 UASM270210	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD211 UASM270211	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD212 UASM270212	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD213 UASM270213	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD214 UASM270214	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD215 UASM270215	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD216 UASM270216	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD217 UASM270217	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD218 UASM270218	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD219 UASM270219	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD220 UASM270220	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD221 UASM270221	*Papilio machaon dodi*	RDR	AB: Tolman Bridge (51.84258, -113.00796) 21.vii.2010; Dupuis, JR	M
JRD268 UASM270268	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 13.viii.2010; Dupuis, JR	M
JRD285 UASM270285	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 13.viii.2010; Dupuis, JR	M
JRD286 UASM270286	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 13.viii.2010; Dupuis, JR	M

JRD485 UASM270485	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD486 UASM270486	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD491 UASM270491	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD492 UASM270492	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD507 UASM270507	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD517 UASM270517	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD728 UASM270728	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD729 UASM270729	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD732 UASM270732	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD735 UASM270735	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD741 UASM270741	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD847 UASM270847	*Papilio machaon dodi*	RDR	AB: Tolman Bridge* (51.84258, -113.00796) 22.viii.2011; Dupuis, JR	M
JRD299 UASM270299	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 18.v.2011; Dupuis, JR	M
JRD305 UASM270305	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	M
JRD308 UASM270308	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 28.v.2011; Dupuis, JR	M
JRD310 UASM270310	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 29.v.2011; Dupuis, JR	M
JRD316 UASM270316	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 30.v.2011; Dupuis, JR	M
JRD567 UASM270567	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	M
JRD570 UASM270570	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	M
JRD572 UASM270572	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	M
JRD575 UASM270575	*Papilio machaon dodi*	RDR	AB: Wintering Hills East (51.25993, -112.45478) 1.vi.2012; Dupuis, JR	M
JRD012 UASM270012	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD013 UASM270013	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z

JRD014 UASM270014	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD015 UASM270015	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD016 UASM270016	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD017 UASM270017	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD018 UASM270018	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 25.v.2010; Dupuis, JR	Z
JRD549 UASM270549	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD550 UASM270550	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD551 UASM270551	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD552 UASM270552	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD553 UASM270553	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD554 UASM270554	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD555 UASM270555	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD556 UASM270556	*Papilio zelicaon x machaon*	FH	AB: Antler Hill (52.06313, -113.89374) 31.v.2012; Dupuis, JR	Z
JRD395 UASM270395	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	Z
JRD396 UASM270396	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	M
JRD397 UASM270397	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	Z
JRD398 UASM270398	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	M
JRD399 UASM270399	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	Z
JRD442 UASM270442	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	Z
JRD444 UASM270444	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	M

JRD445 UASM270445	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	M
JRD714 UASM270714	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 14.vii.2011; Dupuis, JR	M
JRD782 UASM270782	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 18.vii.2008; Dupuis, JR	Z
JRD783 UASM270783	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 18.vii.2008; Dupuis, JR	Z
JRD784 UASM270784	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 18.vii.2008; Dupuis, JR	M
JRD786 UASM270786	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 18.vii.2008; Dupuis, JR	Z
JRD789 UASM270789	*Papilio zelicaon x machaon*	FH	AB: Bragg Creek Ski Hill* (50.981751, -114.58286) 18.vii.2008; Dupuis, JR	H
JRD003 UASM270003	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD004 UASM270004	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD005 UASM270005	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD006 UASM270006	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD007 UASM270007	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD008 UASM270008	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD009 UASM270009	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD010 UASM270010	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD011 UASM270011	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 18.v.2010; Dupuis, JR	Z
JRD022 UASM270022	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2010; Dupuis, JR, Brunet, B	Z
JRD023 UASM270023	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2010; Dupuis, JR, Brunet, B	Z
JRD024 UASM270024	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2010; Dupuis, JR,	Z

			Brunet, B	
JRD025 UASM270025	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2010; Dupuis, JR, Brunet, B	Z
JRD026 UASM270026	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD027 UASM270027	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD028 UASM270028	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD029 UASM270029	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD030 UASM270030	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD031 UASM270031	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD032 UASM270032	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD033 UASM270033	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD034 UASM270034	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 5.vi.2010; Dupuis, JR, Mori, B	Z
JRD102 UASM270102	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 12.vi.2010; Sperling, FAH	Z
JRD103 UASM270103	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 12.vi.2010; Sperling, FAH	Z
JRD104 UASM270104	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 12.vi.2010; Sperling, FAH	Z
JRD105 UASM270105	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 12.vi.2010; Sperling, FAH	Z
JRD106 UASM270106	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 12.vi.2010; Sperling, FAH	Z

JRD238 UASM270238	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD239 UASM270239	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD240 UASM270240	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD241 UASM270241	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD242 UASM270242	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD243 UASM270243	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD244 UASM270244	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD321 UASM270321	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD322 UASM270322	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD323 UASM270323	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD324 UASM270324	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD325 UASM270325	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD326 UASM270326	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD327 UASM270327	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD328 UASM270328	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD329 UASM270329	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD330 UASM270330	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD331 UASM270331	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD332 UASM270332	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD333 UASM270333	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD334 UASM270334	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 31.v.2011; Dupuis, JR	Z
JRD523 UASM270523	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 30.vii.2010; Dupuis, JR	Z
JRD653 UASM270653	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z

JRD654 UASM270654	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD655 UASM270655	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD656 UASM270656	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD657 UASM270657	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD658 UASM270658	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD659 UASM270659	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD660 UASM270660	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD661 UASM270661	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD662 UASM270662	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD663 UASM270663	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD664 UASM270664	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD665 UASM270665	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD666 UASM270666	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD667 UASM270667	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD668 UASM270668	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn* (53.05209, -114.73961) 28.v.2012; Dupuis, JR, Whitehouse, CM	Z
JRD688 UASM270688	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z

JRD689 UASM270689	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD690 UASM270690	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD691 UASM270691	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD692 UASM270692	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD693 UASM270693	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD694 UASM270694	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD695 UASM270695	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	M
JRD696 UASM270696	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD697 UASM270697	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 9.vii.2001; Sperling, FAH	Z
JRD698 UASM270698	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2001; Sperling, FAH	Z
JRD699 UASM270699	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 2.vi.2001; Sperling, FAH	Z
JRD802 UASM270802	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 23.v.2013; McDonald, C	Z
JRD803 UASM270803	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 23.v.2013; McDonald, C	Z
JRD804 UASM270804	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 23.v.2013; Acorn, JH	Z
JRD805 UASM270805	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 23.v.2013; Acorn, JH	Z
JRD806 UASM270806	*Papilio zelicaon x machaon*	FH	AB: Buck Mtn (53.05209, -114.73961) 23.v.2013; Acorn, JH	Z
JRD785 UASM270785	*Papilio zelicaon x machaon*	FH	AB: East of Mesa Butte* (50.765297, -114.371066) 19.vii.2008; Dupuis, JR	Z
JRD866 UASM270866	*Papilio zelicaon x machaon*	FH	AB: East of Mesa Butte* (50.765297, -114.371066) 20.vii.2012; Dupuis, JR	Z
JRD037 UASM270037	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 5.vi.2010; Sperling,	Z

			FAH	
JRD038 UASM270038	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 5.vi.2010; Sperling, FAH	H
JRD039 UASM270039	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 6.vi.2010; Sperling, FAH	M
JRD386 UASM270386	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	M
JRD387 UASM270387	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	M
JRD388 UASM270388	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	Z
JRD389 UASM270389	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	H
JRD390 UASM270390	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	M
JRD391 UASM270391	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	H
JRD392 UASM270392	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	Z
JRD393 UASM270393	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 9.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	M
JRD394 UASM270394	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 10.vii.2011; Sperling, FAH, Sperling, E, Sperling, T	M
JRD581 UASM270581	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 2.vi.2012; Sperling, FAH	M
JRD582 UASM270582	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 2.vi.2012; Sperling, FAH	Z
JRD583 UASM270583	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 2.vi.2012; Sperling, FAH	H
JRD584 UASM270584	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 2.vi.2012; Sperling, FAH	M

JRD585 UASM270585	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 2.vi.2012; Sperling, FAH	M
JRD675 UASM270675	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 6.vii.2002; Sperling, FAH	M
JRD676 UASM270676	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 11.vi.2003; Sperling, FAH	Z
JRD677 UASM270677	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 11.vi.2003; Sperling, FAH	M
JRD678 UASM270678	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 7.vii.2005; Sperling, FAH	Z
JRD679 UASM270679	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 8.vii.2001; Sperling, FAH	Z
JRD680 UASM270680	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 7.vii.2001; Sperling, FAH	Z
JRD681 UASM270681	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 7.vii.2001; Sperling, FAH	H
JRD682 UASM270682	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 7.vii.2001; Sperling, FAH	M
JRD683 UASM270683	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 8.vii.2000; Sperling, FAH	M
JRD684 UASM270684	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 8.vii.2000; Sperling, FAH	Z
JRD685 UASM270685	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 16.viii.2007; Sperling, FAH	M
JRD686 UASM270686	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 8.vii.2001; Sperling, FAH	H
JRD687 UASM270687	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 6.vii.2002; Sperling, FAH	M
JRD700 UASM270700	*Papilio zelicaon x machaon*	FH	AB: Fish Butte (50.91694, -114.535576) 11.vi.2003; Sperling, FAH	Z
JRD702 UASM270702	*Papilio zelicaon x machaon*	FH	AB: Itaska (53.070448, -114.07514) 31.viii.2002; Sperling, FAH	Z

JRD703 UASM270703	*Papilio zelicaon x machaon*	FH	AB: Itaska (53.070448, -114.07514) 21.v.2005; Sperling, FAH	Z
JRD631 UASM270631	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	M
JRD632 UASM270632	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	Z
JRD633 UASM270633	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	M
JRD634 UASM270634	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	M
JRD635 UASM270635	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	Z
JRD636 UASM270636	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	M
JRD637 UASM270637	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	Z
JRD638 UASM270638	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	Z
JRD639 UASM270639	*Papilio zelicaon x machaon*	FH	AB: Jumpingpound Ridge (50.95052, -114.9073) 18.vii.2012; Dupuis, JR	M
JRD204 UASM270204	*Papilio zelicaon x machaon*	FH	AB: Kananaskis Research Station (51.04199, -115.00257) 3.vii.2010; Dupuis, JR, Proshek, B	Z
JRD208 UASM270208	*Papilio zelicaon x machaon*	FH	AB: Kananaskis Research Station (51.02905, -115.035007) 18.vii.2010; Lawrie, D	M
JRD770 UASM270770	*Papilio zelicaon x machaon*	FH	AB: Lloyd Ck* (52.91696, -114.265692) 29.vi.2008; Sperling, FAH	Z
JRD781 UASM270781	*Papilio zelicaon x machaon*	FH	AB: Lloyd Ck* (52.91696, -114.265692) 16.vii.2008; Dupuis, JR	Z
JRD776 UASM270776	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 16.vii.2008; Dupuis, JR	Z
JRD777 UASM270777	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 16.vii.2008; Dupuis, JR	Z
JRD778 UASM270778	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 16.vii.2008; Dupuis, JR	Z
JRD779 UASM270779	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 16.vii.2008; Dupuis, JR	Z
JRD788 UASM270788	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 16.vii.2008; Dupuis, JR	Z

JRD862 UASM270862	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 17.vii.2012; Dupuis, JR	Z
JRD863 UASM270863	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 17.vii.2012; Dupuis, JR	Z
JRD864 UASM270864	*Papilio zelicaon x machaon*	FH	AB: Medicine Lodge Hills* (52.454583, -114.248261) 17.vii.2012; Dupuis, JR	Z
JRD400 UASM270400	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte (50.780713, -114.561342) 15.vii.2011; Dupuis, JR	M
JRD401 UASM270401	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte (50.780713, -114.561342) 15.vii.2011; Dupuis, JR	M
JRD447 UASM270447	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte* (50.780713, -114.561342) 15.vii.2011; Dupuis, JR	H
JRD780 UASM270780	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte* (50.780713, -114.561342) 19.vii.2008; Dupuis, JR	Z
JRD791 UASM270791	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte* (50.780713, -114.561342) 19.vii.2008; Dupuis, JR	Z
JRD871 UASM270871	*Papilio zelicaon x machaon*	FH	AB: Mesa Butte* (50.780713, -114.561342) 20.vii.2012; Dupuis, JR	Z
JRD704 UASM270704	*Papilio zelicaon x machaon*	FH	AB: Moose Mountain (50.93904, -114.8364) 6.vii.2002; Sperling, FAH	M
JRD705 UASM270705	*Papilio zelicaon x machaon*	FH	AB: Moose Mountain (50.93904, -114.8364) 6.vii.2002; Sperling, FAH	Z
JRD706 UASM270706	*Papilio zelicaon x machaon*	FH	AB: Moose Mountain (50.93904, -114.8364) 6.vii.2002; Sperling, FAH	Z
JRD707 UASM270707	*Papilio zelicaon x machaon*	FH	AB: Moose Mountain (50.93904, -114.8364) 7.vii.1991; Sperling, FAH	H
JRD701 UASM270701	*Papilio zelicaon x machaon*	FH	AB: Pigeon Lake Provincial Park (53.027771, -114.150268) 28.vi.2008; Sperling, FAH, Sperling, B	Z
JRD205 UASM270205	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 5.vii.2010; Dupuis, JR	Z
JRD237 UASM270237	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 1.viii.2010; McPike, S	Z
JRD640 UASM270640	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	Z
JRD641 UASM270641	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	H
JRD642 UASM270642	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	Z
JRD643 UASM270643	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	Z
JRD644 UASM270644	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	Z

JRD645 UASM270645	*Papilio zelicaon x machaon*	FH	AB: Powderface Mtn (50.84316, -114.84863) 19.vii.2012; Dupuis, JR	M
JRD193 UASM270193	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD194 UASM270194	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD195 UASM270195	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD196 UASM270196	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD197 UASM270197	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD206 UASM270206	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 9.vii.2010; Dupuis, JR, Mori, B	Z
JRD207 UASM270207	*Papilio zelicaon x machaon*	FH	AB: Shunda Lkt (52.48259, -115.73825) 9.vii.2010; Dupuis, JR, Mori, B	Z
JRD198 UASM270198	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD199 UASM270199	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	M
JRD200 UASM270200	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD201 UASM270201	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	M
JRD202 UASM270202	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	Z
JRD203 UASM270203	*Papilio zelicaon x machaon*	FH	AB: Shunda Mtn (52.53151, -116.1261) 26.vi.2010; Dupuis, JR, Brunet, B	H
JRD796 UASM270796	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 18.v.2013; Acorn, JH	Z
JRD797 UASM270797	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 22.v.2013; Acorn, JH	Z
JRD798 UASM270798	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 23.v.2013; Acorn, JH	Z

JRD799 UASM270799	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 23.v.2013; Dupuis, JR	Z
JRD800 UASM270800	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 23.v.2013; McDonald, C	Z
JRD801 UASM270801	*Papilio zelicaon x machaon*	FH	AB: W of Rimbey (52.63, -114.33) 23.v.2013; McDonald, C	Z
JRD437 UASM270437	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	M
JRD438 UASM270438	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	Z
JRD439 UASM270439	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	M
JRD440 UASM270440	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	Z
JRD441 UASM270441	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	Z
JRD443 UASM270443	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	Z
JRD446 UASM270446	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	Z
JRD448 UASM270448	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	M
JRD713 UASM270713	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 15.vii.2011; Dupuis, JR	M
JRD753 UASM270753	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 18.vii.2012; Dupuis, JR	H
JRD787 UASM270787	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 17.vii.2008; Dupuis, JR	M
JRD865 UASM270865	*Papilio zelicaon x machaon*	FH	AB: Wildcat Hills* (51.28311, -114.67019) 18.vii.2012; Dupuis, JR	Z
JRD335 UASM270335	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 5.vi.2011; Dupuis, JR	Z
JRD337 UASM270337	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD338 UASM270338	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD339 UASM270339	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD340 UASM270340	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD341 UASM270341	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD342 UASM270342	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD343 UASM270343	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z

JRD344 UASM270344	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD345 UASM270345	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD346 UASM270346	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD347 UASM270347	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD348 UASM270348	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD349 UASM270349	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD350 UASM270350	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD351 UASM270351	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD352 UASM270352	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD353 UASM270353	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD354 UASM270354	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD355 UASM270355	*Papilio zelicaon*	SH	AB: Enilda Lkt (55.31975, -116.19717) 7.vi.2011; Dupuis, JR	Z
JRD054 UASM270054	*Papilio zelicaon*	SH	AB: Flattop Lkt (55.14584, -114.81561) 10.vi.2010; Dupuis, JR	Z
JRD055 UASM270055	*Papilio zelicaon*	SH	AB: Flattop Lkt (55.14584, -114.81561) 10.vi.2010; Dupuis, JR	Z
JRD056 UASM270056	*Papilio zelicaon*	SH	AB: Flattop Lkt (55.14584, -114.81561) 10.vi.2010; Dupuis, JR	Z
JRD057 UASM270057	*Papilio zelicaon*	SH	AB: Flattop Lkt (55.14584, -114.81561) 10.vi.2010; Dupuis, JR	Z
JRD058 UASM270058	*Papilio zelicaon*	SH	AB: Flattop Lkt (55.14584, -114.81561) 10.vi.2010; Dupuis, JR	Z
JRD425 UASM270425	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 1.vii.2011; Dupuis, JR	Z
JRD430 UASM270430	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 1.vii.2011; Dupuis, JR	Z
JRD434 UASM270434	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 1.vii.2011; Dupuis, JR	Z
JRD435 UASM270435	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 1.vii.2011; Dupuis, JR	Z
JRD710 UASM270710	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 1.vii.2011; Dupuis, JR	Z
JRD858 UASM270858	*Papilio zelicaon*	SH	AB: Fox Creek* (54.36023, -116.70983) 21.vi.2012; Dupuis, JR	Z

JRD076 UASM270076	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD077 UASM270077	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD078 UASM270078	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD079 UASM270079	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD080 UASM270080	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD081 UASM270081	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD082 UASM270082	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD083 UASM270083	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD084 UASM270084	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD085 UASM270085	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD086 UASM270086	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD087 UASM270087	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD088 UASM270088	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD089 UASM270089	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD090 UASM270090	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD091 UASM270091	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD092 UASM270092	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD093 UASM270093	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD094 UASM270094	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD095 UASM270095	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD096 UASM270096	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD097 UASM270097	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD098 UASM270098	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z

JRD099 UASM270099	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD100 UASM270100	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD101 UASM270101	*Papilio zelicaon*	SH	AB: Goose Mtn (54.75171, -116.03276) 12.vi.2010; Dupuis, JR	Z
JRD059 UASM270059	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD060 UASM270060	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD061 UASM270061	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD062 UASM270062	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD063 UASM270063	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD064 UASM270064	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD065 UASM270065	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD066 UASM270066	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD067 UASM270067	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD068 UASM270068	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD069 UASM270069	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD070 UASM270070	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD071 UASM270071	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD072 UASM270072	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD073 UASM270073	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD074 UASM270074	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD075 UASM270075	*Papilio zelicaon*	SH	AB: House Mtn (55.04839, -115.59475) 11.vi.2010; Dupuis, JR	Z
JRD035 UASM270035	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD036 UASM270036	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD040 UASM270040	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z

JRD041 UASM270041	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD042 UASM270042	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD043 UASM270043	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD044 UASM270044	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD045 UASM270045	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD046 UASM270046	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD048 UASM270048	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD049 UASM270049	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD050 UASM270050	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD051 UASM270051	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD052 UASM270052	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD053 UASM270053	*Papilio zelicaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	Z
JRD356 UASM270356	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD357 UASM270357	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD358 UASM270358	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD359 UASM270359	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD360 UASM270360	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD361 UASM270361	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD362 UASM270362	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD363 UASM270363	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD364 UASM270364	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 7.vi.2011; Dupuis, JR	Z
JRD365 UASM270365	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD366 UASM270366	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z

JRD367 UASM270367	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD368 UASM270368	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD369 UASM270369	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD370 UASM270370	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD371 UASM270371	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD372 UASM270372	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD417 UASM270417	*Papilio zelicaon*	SH	AB: Pushwaskau Lkt* (55.218752, -117.493001) 8.vi.2011; Dupuis, JR	Z
JRD373 UASM270373	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD374 UASM270374	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD375 UASM270375	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD376 UASM270376	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD377 UASM270377	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD378 UASM270378	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD379 UASM270379	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD380 UASM270380	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD381 UASM270381	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD382 UASM270382	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD383 UASM270383	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD384 UASM270384	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD385 UASM270385	*Papilio zelicaon*	SH	AB: Sweathouse Lkt (54.89917, -116.75206) 8.vi.2011; Dupuis, JR	Z
JRD586 UASM270586	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD587 UASM270587	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD588 UASM270588	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z

JRD589 UASM270589	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD590 UASM270590	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD591 UASM270591	*Papilio zelicaon*	SH	AB: Whitecourt Mtn (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD755 UASM270755	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 6.vi.2008; Dupuis, JR	Z
JRD756 UASM270756	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 6.vi.2008; Dupuis, JR	Z
JRD757 UASM270757	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 6.vi.2008; Dupuis, JR	Z
JRD758 UASM270758	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 6.vi.2008; Dupuis, JR	Z
JRD759 UASM270759	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 6.vi.2008; Dupuis, JR	Z
JRD849 UASM270849	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD850 UASM270850	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD851 UASM270851	*Papilio zelicaon*	SH	AB: Whitecourt Mtn* (54.03232, -115.72055) 7.vi.2012; Dupuis, JR	Z
JRD047 UASM270047	*Papilio zelicaon x machaon*	SH	AB: Marten Mtn (55.47031, -114.78509) 10.vi.2010; Dupuis, JR	H
JRD451 UASM270451	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD455 UASM270455	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD456 UASM270456	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD457 UASM270457	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD463 UASM270463	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD471 UASM270471	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD475 UASM270475	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD717 UASM270717	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD724 UASM270724	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD725 UASM270725	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	M
JRD874 UASM270874	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 1.viii.2012; Dupuis, JR	M

JRD875 UASM270875	*Papilio machaon pikei*	PR	BC: Beatton River* (56.27525, -120.67391) 1.viii.2012; Dupuis, JR	M
JRD119 UASM270119	*Papilio machaon pikei*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	M
JRD466 UASM270466	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 13.viii.2011; Dupuis, JR	M
JRD467 UASM270467	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 13.viii.2011; Dupuis, JR	M
JRD488 UASM270488	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 13.viii.2011; Dupuis, JR	M
JRD722 UASM270722	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 13.viii.2011; Dupuis, JR	M
JRD790 UASM270790	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 1.viii.2008; Dupuis, JR	M
JRD873 UASM270873	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 2.viii.2012; Dupuis, JR	M
JRD877 UASM270877	*Papilio machaon pikei*	PR	BC: Clayhurst Ferry* (56.12865, -120.05228) 2.viii.2012; Dupuis, JR	M
JRD465 UASM270465	*Papilio machaon pikei*	PR	AB: Dunvegan* (55.92534, -118.60135) 14.viii.2011; Dupuis, JR	M
JRD472 UASM270472	*Papilio machaon pikei*	PR	AB: Dunvegan* (55.92534, -118.60135) 14.viii.2011; Dupuis, JR	M
JRD795 UASM270795	*Papilio machaon pikei*	PR	AB: Dunvegan* (55.92534, -118.60135) 1.viii.2008; Dupuis, JR	M
JRD621 UASM270621	*Papilio machaon pikei*	PR	AB: Highland Park Central (56.01923, -118.80972) 21.vi.2012; Dupuis, JR	M
JRD622 UASM270622	*Papilio machaon pikei*	PR	AB: Highland Park Central (56.01923, -118.80972) 21.vi.2012; Dupuis, JR	M
JRD828 UASM270828	*Papilio machaon pikei*	PR	AB: Highland Park Central (56.01923, -118.80972) 15.vii.2013; Dupuis, JR	M
JRD170 UASM270170	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD171 UASM270171	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD172 UASM270172	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD173 UASM270173	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD174 UASM270174	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD175 UASM270175	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD176 UASM270176	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M

JRD177 UASM270177	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD178 UASM270178	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 19.vi.2010; Dupuis, JR	M
JRD616 UASM270616	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 20.vi.2012; Dupuis, JR	M
JRD617 UASM270617	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 20.vi.2012; Dupuis, JR	M
JRD618 UASM270618	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 21.vi.2012; Dupuis, JR	M
JRD619 UASM270619	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 21.vi.2012; Dupuis, JR	M
JRD620 UASM270620	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 21.vi.2012; Dupuis, JR	M
JRD816 UASM270816	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD817 UASM270817	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD818 UASM270818	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD819 UASM270819	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD820 UASM270820	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD821 UASM270821	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD822 UASM270822	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD823 UASM270823	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD824 UASM270824	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD825 UASM270825	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD826 UASM270826	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD827 UASM270827	*Papilio machaon pikei*	PR	AB: Highland Park North (56.13076, -118.88931) 15.vii.2013; Dupuis, JR	M
JRD180 UASM270180	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD181 UASM270181	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD182 UASM270182	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD183 UASM270183	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M

JRD185 UASM270185	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD186 UASM270186	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD187 UASM270187	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD188 UASM270188	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD189 UASM270189	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD190 UASM270190	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD191 UASM270191	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD192 UASM270192	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	M
JRD807 UASM270807	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 4.vii.2013; Dupuis, JR	M
JRD808 UASM270808	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 4.vii.2013; Dupuis, JR	M
JRD809 UASM270809	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 4.vii.2013; Dupuis, JR	M
JRD811 UASM270811	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 14.vii.2013; Dupuis, JR	M
JRD812 UASM270812	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 14.vii.2013; Dupuis, JR	M
JRD813 UASM270813	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 14.vii.2013; Dupuis, JR	M
JRD814 UASM270814	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 14.vii.2013; Dupuis, JR	M
JRD815 UASM270815	*Papilio machaon pikei*	PR	AB: Kaufman Hill (56.24883, -117.27408) 14.vii.2013; Dupuis, JR	M
JRD123 UASM270123	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD124 UASM270124	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD125 UASM270125	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD127 UASM270127	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD129 UASM270129	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD130 UASM270130	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD131 UASM270131	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M

JRD132 UASM270132	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD133 UASM270133	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD134 UASM270134	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD135 UASM270135	*Papilio machaon pikei*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	M
JRD478 UASM270478	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD489 UASM270489	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD493 UASM270493	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD495 UASM270495	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD498 UASM270498	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD506 UASM270506	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD716 UASM270716	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD720 UASM270720	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD721 UASM270721	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD723 UASM270723	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD726 UASM270726	*Papilio machaon pikei*	PR	BC: Lynx Ck* (56.11653, -121.79996) 13.viii.2011; Dupuis, JR	M
JRD792 UASM270792	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 1.viii.2008; Dupuis, JR	M
JRD829 UASM270829	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD830 UASM270830	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD831 UASM270831	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD832 UASM270832	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD833 UASM270833	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD834 UASM270834	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD835 UASM270835	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M

JRD836 UASM270836	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD837 UASM270837	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD838 UASM270838	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD839 UASM270839	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD840 UASM270840	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD841 UASM270841	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD842 UASM270842	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD843 UASM270843	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD844 UASM270844	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry (56.121675, -117.415466) 17.vii.2013; Dupuis, JR	M
JRD868 UASM270868	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 2.viii.2012; Dupuis, JR	M
JRD869 UASM270869	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 2.viii.2012; Dupuis, JR	M
JRD870 UASM270870	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 2.viii.2012; Dupuis, JR	M
JRD872 UASM270872	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 2.viii.2012; Dupuis, JR	M
JRD876 UASM270876	*Papilio machaon pikei*	PR	AB: Shaftsbury Ferry* (56.121675, -117.415466) 2.viii.2012; Dupuis, JR	M
JRD449 UASM270449	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD450 UASM270450	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD452 UASM270452	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD453 UASM270453	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD454 UASM270454	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD460 UASM270460	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD461 UASM270461	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD462 UASM270462	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD464 UASM270464	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M

JRD468 UASM270468	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD469 UASM270469	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD470 UASM270470	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD473 UASM270473	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD474 UASM270474	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD476 UASM270476	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD477 UASM270477	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD479 UASM270479	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD480 UASM270480	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD481 UASM270481	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD482 UASM270482	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD484 UASM270484	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD715 UASM270715	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD718 UASM270718	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD719 UASM270719	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD793 UASM270793	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 31.vii.2008; Dupuis, JR	M
JRD794 UASM270794	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 31.vii.2008; Dupuis, JR	M
JRD846 UASM270846	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD867 UASM270867	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 1.viii.2012; Dupuis, JR	M
JRD878 UASM270878	*Papilio machaon pikei*	PR	BC: Taylor* (56.15403, -120.71828) 1.viii.2012; Dupuis, JR	M
JRD458 UASM270458	*Papilio machaon pikei*	PR	BC: Taylor * (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD459 UASM270459	*Papilio machaon pikei*	PR	BC: Taylor * (56.15403, -120.71828) 11.viii.2011; Dupuis, JR	M
JRD406 UASM270406	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z

JRD407 UASM270407	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD408 UASM270408	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD409 UASM270409	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD410 UASM270410	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD411 UASM270411	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD412 UASM270412	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD413 UASM270413	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD436 UASM270436	*Papilio zelicaon*	PR	BC: Bear Mtn* (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD601 UASM270601	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD602 UASM270602	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD603 UASM270603	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD604 UASM270604	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD605 UASM270605	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD606 UASM270606	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD607 UASM270607	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD608 UASM270608	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD609 UASM270609	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD610 UASM270610	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD611 UASM270611	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD612 UASM270612	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD613 UASM270613	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD614 UASM270614	*Papilio zelicaon*	PR	BC: Bear Mtn (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD766 UASM270766	*Papilio zelicaon*	PR	BC: Bear Mtn* (55.72849, -120.44299) 19.vi.2008; Dupuis, JR	Z

JRD845 UASM270845	*Papilio zelicaon*	PR	BC: Bear Mtn* (55.72849, -120.44299) 30.vi.2011; Dupuis, JR	Z
JRD853 UASM270853	*Papilio zelicaon*	PR	BC: Bear Mtn* (55.72849, -120.44299) 20.vi.2012; Dupuis, JR	Z
JRD483 UASM270483	*Papilio zelicaon*	PR	BC: Beatton River* (56.27525, -120.67391) 14.viii.2011; Dupuis, JR	Z
JRD108 UASM270108	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD109 UASM270109	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD110 UASM270110	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD111 UASM270111	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD112 UASM270112	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD113 UASM270113	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD114 UASM270114	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD115 UASM270115	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD116 UASM270116	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD117 UASM270117	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD118 UASM270118	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD120 UASM270120	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD121 UASM270121	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD122 UASM270122	*Papilio zelicaon*	PR	BC: Bullhead Mtn (56.0457, -122.13297) 17.vi.2010; Dupuis, JR	Z
JRD179 UASM270179	*Papilio zelicaon*	PR	AB: Highland Park Central (56.01923, -118.80972) 19.vi.2010; Dupuis, JR	Z
JRD184 UASM270184	*Papilio zelicaon*	PR	AB: Kaufman Hill (56.24883, -117.27408) 19.vi.2010; Dupuis, JR	Z
JRD107 UASM270107	*Papilio zelicaon*	PR	AB: Kleskun Hills (55.25466, -118.52737) 16.vi.2010; Dupuis, JR	Z
JRD760 UASM270760	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD761 UASM270761	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD762 UASM270762	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z

JRD763 UASM270763	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD764 UASM270764	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD765 UASM270765	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD768 UASM270768	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD772 UASM270772	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 18.vi.2008; Dupuis, JR	Z
JRD852 UASM270852	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 19.vi.2012; Dupuis, JR	Z
JRD854 UASM270854	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 19.vi.2012; Dupuis, JR	Z
JRD855 UASM270855	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 19.vi.2012; Dupuis, JR	Z
JRD860 UASM270860	*Papilio zelicaon*	PR	AB: Kleskun Hills* (55.25466, -118.52737) 19.vi.2012; Dupuis, JR	Z
JRD126 UASM270126	*Papilio zelicaon*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	Z
JRD128 UASM270128	*Papilio zelicaon*	PR	BC: Lynx Ck (56.11653, -121.79996) 17.vi.2010; Dupuis, JR	Z
JRD615 UASM270615	*Papilio zelicaon*	PR	BC: Pouce Coupe (55.72493, -120.05585) 20.vi.2012; Dupuis, JR	Z
JRD767 UASM270767	*Papilio zelicaon*	PR	BC: Pouce Coupe* (55.72493, -120.05585) 19.vi.2008; Dupuis, JR	Z
JRD402 UASM270402	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD403 UASM270403	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD404 UASM270404	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD405 UASM270405	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD418 UASM270418	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD419 UASM270419	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD420 UASM270420	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD421 UASM270421	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD422 UASM270422	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD423 UASM270423	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z

JRD424 UASM270424	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD426 UASM270426	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD427 UASM270427	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD428 UASM270428	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD429 UASM270429	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD433 UASM270433	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.22422, -119.29686) 29.vi.2011; Dupuis, JR	Z
JRD595 UASM270595	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD596 UASM270596	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD597 UASM270597	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD598 UASM270598	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD599 UASM270599	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD600 UASM270600	*Papilio zelicaon*	PR	AB: Saskatoon Hill (55.22422, -119.29686) 19.vi.2012; Dupuis, JR	Z
JRD711 UASM270711	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.218752, -117.493001) 29.vi.2011; Dupuis, JR	Z
JRD712 UASM270712	*Papilio zelicaon*	PR	AB: Saskatoon Hill* (55.218752, -117.493001) 29.vi.2011; Dupuis, JR	Z
JRD336 UASM270336	*Papilio zelicaon*	PR	AB: White Mtn (55.6943, -119.23857) 6.vi.2011; Dupuis, JR	Z
JRD431 UASM270431	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 1.vii.2011; Dupuis, JR	Z
JRD432 UASM270432	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 1.vii.2011; Dupuis, JR	Z
JRD623 UASM270623	*Papilio zelicaon*	PR	AB: White Mtn (55.6943, -119.23857) 21.vi.2012; Dupuis, JR	Z
JRD769 UASM270769	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 20.vi.2008; Dupuis, JR	Z
JRD771 UASM270771	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 20.vi.2008; Dupuis, JR	Z
JRD773 UASM270773	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 20.vi.2008; Dupuis, JR	Z
JRD774 UASM270774	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 20.vi.2008; Dupuis, JR	Z
JRD775 UASM270775	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 20.vi.2008; Dupuis, JR	Z

JRD856 UASM270856	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 21.vi.2012; Dupuis, JR	Z
JRD857 UASM270857	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 21.vi.2012; Dupuis, JR	Z
JRD859 UASM270859	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 21.vi.2012; Dupuis, JR	Z
JRD861 UASM270861	*Papilio zelicaon*	PR	AB: White Mtn* (55.6943, -119.23857) 21.vi.2012; Dupuis, JR	Z
JRD810 UASM270810	*Papilio zelicaon x machaon*	PR	AB: Kaufman Hill (56.24883, -117.27408) 4.vii.2013; Dupuis, JR	H

Appendix 4.2 Pairwise F_{ST} values for all locality samples with >7 individuals. Lower triangle: normal F_{ST}, bolded values indicate non-significant comparisons after Bonferroni correction ($p > 0.05$). Upper triangle: F_{ST} corrected for null alleles.

	1	2	3	4	5	6	7	8
Hand Hills (1)		0.007	-0.003	0.102	0.132	0.098	0.112	0.095
Wintering Hills E (2)	**0.007**		0.010	0.052	0.076	0.056	0.060	0.049
Wintering Hills W (3)	**-0.003**	**0.012**		0.108	0.131	0.109	0.111	0.098
Horsethief Canyon (4)	0.122	0.065	0.129		0.000	0.001	0.006	-0.002
Lousana (5)	0.141	0.082	0.142	**-0.002**		0.013	0.014	0.011
Morrin Bridge (6)	0.124	0.069	0.131	**0.001**	**0.014**		0.005	0.009
North Drumheller (7)	0.133	0.069	0.132	**0.002**	**0.012**	**0.002**		0.006
Orkney Lookout (8)	0.114	0.061	0.120	**-0.002**	**0.012**	**0.010**	**0.003**	
Tolman Bridge (9)	0.135	0.071	0.132	**0.000**	**0.002**	**0.005**	**0.005**	**0.010**
Antler Hill (10)	**0.007**	**0.022**	**0.020**	0.131	0.147	0.138	0.145	0.137
Bragg Creek Ski Hill (11)	**0.028**	0.043	0.040	0.134	0.145	0.144	0.151	0.131
Buck Mtn (12)	**0.017**	0.031	0.030	0.127	0.140	0.136	0.144	0.128
Fish Butte (13)	**0.030**	0.034	0.042	0.104	0.120	0.109	0.115	0.100
Jumpingpound Ridge (14)	**0.010**	**0.026**	**0.020**	0.116	0.133	0.125	0.120	0.113
Medicine Hills (15)	**0.025**	**0.028**	**0.042**	0.140	0.159	0.152	0.149	0.146
Mesa Butte (16)	**0.014**	**0.024**	**0.036**	0.116	0.141	0.123	0.139	0.116
Powderface Ridge (17)	**-0.002**	**0.017**	**0.016**	0.113	0.136	0.124	0.127	0.111
Shunda Mtn (18)	**0.016**	**0.029**	0.029	0.121	0.135	0.128	0.140	0.122
Wildcat Hills (19)	**0.030**	0.033	0.042	0.109	0.131	0.114	0.112	0.109
Enilda Lookout (20)	**0.016**	0.039	0.031	0.150	0.167	0.158	0.163	0.147
Goose Mtn (21)	**0.024**	0.036	0.034	0.142	0.157	0.151	0.158	0.142
House Mtn (22)	**0.020**	**0.022**	0.032	0.127	0.143	0.136	0.140	0.125
Marten Mtn (23)	**0.031**	0.037	0.038	0.148	0.165	0.159	0.163	0.151
Pushwaskau Lookout (24)	**0.019**	0.031	0.037	0.137	0.151	0.147	0.155	0.136
Sweathouse Lookout (25)	**0.003**	**0.017**	**0.020**	0.131	0.146	0.137	0.137	0.123
Whitecourt Mtn (26)	**0.013**	0.033	0.035	0.141	0.157	0.147	0.152	0.142
Beatton River (27)	0.275	0.215	0.265	0.210	0.221	0.236	0.235	0.207
Clayhurst (28)	**0.198**	0.159	0.204	0.148	0.167	0.175	0.171	0.142
Highland Park (29)	0.203	0.151	0.206	0.131	0.142	0.151	0.147	0.119
Kaufman Hill (30)	0.211	0.167	0.216	0.151	0.172	0.173	0.178	0.148
Lynx Ridge (31)	0.214	0.177	0.223	0.185	0.197	0.199	0.201	0.184
Shaftsbury Trail (32)	0.255	0.204	0.250	0.194	0.209	0.221	0.226	0.189
Taylor (33)	0.292	0.225	0.283	0.203	0.218	0.231	0.231	0.198
Bear Mtn (34)	**0.031**	0.040	0.040	0.150	0.162	0.155	0.166	0.152
Bullhead Mtn (35)	**0.013**	**0.022**	0.027	0.139	0.153	0.144	0.154	0.139
Kleskun Hills (36)	**0.041**	0.051	0.059	0.150	0.168	0.159	0.176	0.152
Saskatoon Mtn (37)	**0.019**	0.034	0.029	0.156	0.169	0.160	0.166	0.153
White Mtn (38)	**0.026**	**0.034**	0.037	0.146	0.168	0.157	0.171	0.150

	9	10	11	12	13	14	15	16
Hand Hills (1)	0.120	0.009	0.027	0.015	0.027	0.013	0.022	0.017
Wintering Hills E (2)	0.062	0.022	0.037	0.027	0.030	0.026	0.023	0.027
Wintering Hills W (3)	0.115	0.021	0.038	0.028	0.039	0.022	0.029	0.037
Horsethief Canyon (4)	0.003	0.109	0.103	0.099	0.079	0.093	0.113	0.098
Lousana (5)	0.002	0.136	0.129	0.120	0.106	0.125	0.144	0.133
Morrin Bridge (6)	0.007	0.111	0.109	0.107	0.082	0.096	0.117	0.098
North Drumheller (7)	0.007	0.123	0.120	0.119	0.092	0.095	0.118	0.119
Orkney Lookout (8)	0.009	0.115	0.107	0.099	0.076	0.095	0.125	0.106
Tolman Bridge (9)		0.125	0.125	0.116	0.104	0.116	0.135	0.128
Antler Hill (10)	0.143		0.035	0.000	0.032	0.018	0.000	0.043
Bragg Creek Ski Hill (11)	0.150	**0.036**		0.027	0.013	0.001	0.033	0.014
Buck Mtn (12)	0.141	**-0.003**	0.029		0.023	0.025	0.010	0.022
Fish Butte (13)	0.125	0.031	**0.015**	0.026		0.005	0.032	-0.004
Jumpingpound Ridge (14)	0.134	**0.014**	**-0.004**	**0.022**	**0.000**		0.033	0.012
Medicine Hills (15)	0.159	**-0.001**	**0.033**	**0.009**	**0.034**	**0.033**		0.037
Mesa Butte (16)	0.144	**0.040**	**0.012**	**0.019**	**-0.008**	**0.001**	**0.036**	
Powderface Ridge (17)	0.136	**0.007**	**0.008**	**0.000**	**-0.002**	**-0.015**	**0.029**	**-0.008**
Shunda Mtn (18)	0.134	**0.009**	**0.017**	**0.007**	**0.010**	**0.000**	**0.022**	**0.000**
Wildcat Hills (19)	0.134	**0.026**	**0.023**	0.033	**0.000**	**0.012**	**0.023**	**0.016**
Enilda Lookout (20)	0.163	**-0.003**	0.046	**0.005**	0.041	**0.016**	**0.021**	**0.039**
Goose Mtn (21)	0.157	**-0.005**	0.035	**-0.002**	0.029	**0.017**	**0.002**	**0.029**
House Mtn (22)	0.143	**0.004**	**0.014**	**0.001**	**0.012**	**0.018**	**-0.008**	**0.016**
Marten Mtn (23)	0.160	**0.008**	**0.031**	**0.009**	0.037	**0.024**	**0.019**	**0.037**
Pushwaskau Lookout (24)	0.154	**0.006**	**0.029**	**0.000**	0.023	**0.023**	**-0.004**	**0.013**
Sweathouse Lookout (25)	0.140	**-0.007**	**0.030**	**0.000**	**0.026**	**0.009**	**0.005**	**0.031**
Whitecourt Mtn (26)	0.158	**0.001**	**0.041**	**0.004**	0.039	**0.024**	**0.010**	**0.032**
Beatton River (27)	0.231	0.258	0.265	0.222	0.219	0.270	0.292	0.281
Clayhurst (28)	0.178	0.199	0.201	0.176	0.162	**0.193**	**0.218**	**0.192**
Highland Park (29)	0.150	0.196	0.200	0.172	0.164	0.193	0.205	0.198
Kaufman Hill (30)	0.179	0.204	0.221	0.182	0.176	0.210	0.231	0.216
Lynx Ridge (31)	0.207	0.206	0.216	0.176	0.174	0.213	0.214	0.211
Shaftsbury Trail (32)	0.217	0.254	0.260	0.217	0.219	0.261	0.282	0.266
Taylor (33)	0.226	0.274	0.282	0.228	0.228	0.283	0.302	0.284
Bear Mtn (34)	0.160	**0.010**	0.050	**0.010**	0.045	**0.034**	**0.012**	0.047
Bullhead Mtn (35)	0.154	**0.003**	**0.030**	**-0.001**	0.027	**0.019**	**0.005**	**0.020**
Kleskun Hills (36)	0.167	**0.010**	0.053	**0.019**	0.049	**0.043**	**-0.002**	**0.048**
Saskatoon Mtn (37)	0.166	**0.003**	0.036	**0.006**	0.040	**0.034**	**0.009**	**0.036**
White Mtn (38)	0.165	**0.007**	**0.047**	**0.009**	0.034	**0.040**	**0.005**	**0.024**

	17	18	19	20	21	22	23	24
Hand Hills (1)	-0.003	0.017	0.029	0.017	0.023	0.022	0.030	0.020
Wintering Hills E (2)	0.017	0.031	0.034	0.036	0.034	0.024	0.037	0.030
Wintering Hills W (3)	0.015	0.032	0.042	0.028	0.033	0.034	0.038	0.037
Horsethief Canyon (4)	0.091	0.098	0.091	0.123	0.116	0.104	0.123	0.114
Lousana (5)	0.126	0.121	0.122	0.150	0.142	0.132	0.152	0.139
Morrin Bridge (6)	0.093	0.103	0.092	0.127	0.122	0.110	0.130	0.121
North Drumheller (7)	0.101	0.120	0.093	0.136	0.133	0.119	0.139	0.133
Orkney Lookout (8)	0.091	0.103	0.092	0.121	0.117	0.106	0.126	0.115
Tolman Bridge (9)	0.118	0.117	0.120	0.141	0.135	0.125	0.140	0.135
Antler Hill (10)	0.009	0.012	0.027	-0.002	-0.002	0.008	0.010	0.008
Bragg Creek Ski Hill (11)	0.007	0.021	0.020	0.041	0.034	0.018	0.032	0.032
Buck Mtn (12)	0.003	0.007	0.032	0.004	0.000	0.004	0.010	0.002
Fish Butte (13)	0.000	0.013	0.002	0.037	0.029	0.015	0.039	0.025
Jumpingpound Ridge (14)	-0.009	0.009	0.013	0.022	0.022	0.024	0.029	0.030
Medicine Hills (15)	0.026	0.022	0.018	0.019	0.001	-0.002	0.015	0.006
Mesa Butte (16)	-0.004	0.008	0.015	0.040	0.030	0.021	0.037	0.018
Powderface Ridge (17)		-0.004	0.004	0.013	0.016	0.013	0.016	0.006
Shunda Mtn (18)	**-0.008**		0.019	0.020	0.004	0.003	0.008	0.006
Wildcat Hills (19)	**0.008**	**0.018**		0.040	0.034	0.019	0.043	0.028
Enilda Lookout (20)	**0.011**	**0.020**	0.043		0.003	0.015	0.009	0.016
Goose Mtn (21)	**0.016**	**0.004**	0.035	**0.002**		-0.004	0.004	0.000
House Mtn (22)	**0.011**	**0.000**	**0.018**	**0.014**	**-0.006**		0.014	-0.001
Marten Mtn (23)	**0.019**	**0.006**	0.043	**0.007**	**0.001**	**0.012**		0.016
Pushwaskau Lookout (24)	**0.002**	**0.004**	**0.028**	**0.014**	**-0.001**	**-0.002**	**0.015**	
Sweathouse Lookout (25)	**0.002**	**0.013**	**0.032**	**-0.010**	**0.004**	**0.002**	**0.006**	**-0.002**
Whitecourt Mtn (26)	**0.016**	**0.024**	0.045	**0.004**	**0.001**	**0.007**	**0.021**	**0.008**
Beatton River (27)	0.252	0.239	0.232	0.271	0.242	0.237	0.271	0.238
Clayhurst (28)	**0.177**	0.176	0.168	0.207	0.188	0.174	0.207	0.181
Highland Park (29)	0.179	0.182	0.171	0.207	0.186	0.174	0.205	0.179
Kaufman Hill (30)	0.191	0.200	0.186	0.218	0.200	0.192	0.221	0.202
Lynx Ridge (31)	0.178	0.181	0.177	0.221	0.196	0.184	0.223	0.178
Shaftsbury Trail (32)	0.256	0.253	0.237	0.255	0.241	0.240	0.259	0.244
Taylor (33)	0.263	0.254	0.243	0.287	0.256	0.249	0.286	0.253
Bear Mtn (34)	**0.030**	**0.020**	0.049	**0.005**	**0.002**	**0.003**	**0.024**	**0.004**
Bullhead Mtn (35)	**0.016**	**0.011**	0.037	**0.008**	**-0.008**	**-0.009**	**0.013**	**-0.002**
Kleskun Hills (36)	**0.045**	**0.018**	0.059	**0.014**	**0.005**	**0.020**	**0.022**	**0.017**
Saskatoon Mtn (37)	**0.029**	**0.021**	0.043	**0.011**	**0.004**	**-0.004**	0.030	**0.009**
White Mtn (38)	**0.014**	**0.011**	0.042	**0.019**	**0.002**	**0.004**	**0.020**	**0.006**

	25	26	27	28	29	30	31	32
Hand Hills (1)	0.006	0.012	0.267	0.183	0.179	0.188	0.200	0.250
Wintering Hills E (2)	0.016	0.031	0.215	0.153	0.135	0.154	0.172	0.203
Wintering Hills W (3)	0.017	0.032	0.259	0.190	0.184	0.196	0.210	0.245
Horsethief Canyon (4)	0.107	0.118	0.198	0.134	0.115	0.139	0.179	0.185
Lousana (5)	0.135	0.146	0.209	0.152	0.129	0.158	0.195	0.199
Morrin Bridge (6)	0.110	0.119	0.220	0.157	0.126	0.152	0.187	0.203
North Drumheller (7)	0.114	0.128	0.225	0.158	0.132	0.164	0.198	0.215
Orkney Lookout (8)	0.101	0.121	0.193	0.128	0.104	0.135	0.182	0.179
Tolman Bridge (9)	0.121	0.140	0.214	0.159	0.131	0.163	0.201	0.201
Antler Hill (10)	-0.004	0.002	0.251	0.184	0.174	0.182	0.194	0.248
Bragg Creek Ski Hill (11)	0.025	0.038	0.260	0.188	0.173	0.192	0.205	0.252
Buck Mtn (12)	0.001	0.006	0.221	0.165	0.153	0.166	0.168	0.213
Fish Butte (13)	0.023	0.038	0.217	0.151	0.143	0.157	0.166	0.213
Jumpingpound Ridge (14)	0.015	0.026	0.265	0.182	0.172	0.187	0.205	0.255
Medicine Hills (15)	0.004	0.004	0.281	0.203	0.179	0.202	0.206	0.274
Mesa Butte (16)	0.030	0.032	0.276	0.184	0.181	0.196	0.206	0.267
Powderface Ridge (17)	0.005	0.015	0.243	0.160	0.157	0.166	0.169	0.246
Shunda Mtn (18)	0.011	0.024	0.238	0.170	0.163	0.181	0.171	0.252
Wildcat Hills (19)	0.030	0.043	0.221	0.149	0.148	0.162	0.165	0.229
Enilda Lookout (20)	-0.005	0.006	0.265	0.193	0.185	0.195	0.208	0.247
Goose Mtn (21)	0.003	0.001	0.239	0.176	0.167	0.181	0.185	0.237
House Mtn (22)	0.004	0.008	0.235	0.163	0.153	0.173	0.173	0.237
Marten Mtn (23)	0.008	0.019	0.261	0.189	0.181	0.197	0.209	0.254
Pushwaskau Lookout (24)	0.002	0.009	0.237	0.169	0.161	0.184	0.171	0.241
Sweathouse Lookout (25)		0.006	0.243	0.175	0.163	0.184	0.188	0.242
Whitecourt Mtn (26)	**0.003**		0.258	0.181	0.171	0.183	0.196	0.247
Beatton River (27)	0.251	0.264		0.006	0.043	0.049	0.040	0.082
Clayhurst (28)	0.189	0.198	**0.005**		-0.005	-0.006	0.021	0.025
Highland Park (29)	0.186	0.193	**0.033**	**-0.015**		0.011	0.047	0.047
Kaufman Hill (30)	0.206	0.206	**0.051**	**-0.008**	**0.009**		0.051	0.036
Lynx Ridge (31)	0.202	0.207	**0.021**	**0.007**	**0.038**	0.048		0.129
Shaftsbury Trail (32)	0.248	0.251	0.101	**0.034**	**0.045**	**0.036**	0.128	
Taylor (33)	0.270	0.279	**-0.007**	**-0.004**	**0.024**	**0.031**	**0.037**	0.084
Bear Mtn (34)	**-0.002**	**0.011**	0.271	0.213	0.201	0.221	0.219	0.259
Bullhead Mtn (35)	**-0.003**	**-0.009**	0.262	0.199	0.191	0.211	0.210	0.252
Kleskun Hills (36)	**0.014**	**0.031**	0.286	0.211	0.202	0.217	0.229	0.257
Saskatoon Mtn (37)	**0.002**	**0.012**	0.267	0.212	0.209	0.224	0.215	0.263
White Mtn (38)	**0.008**	**0.011**	0.285	0.214	0.208	0.220	0.229	0.271

	33	34	35	36	37	38
Hand Hills (1)	0.278	0.026	0.012	0.034	0.020	0.023
Wintering Hills E (2)	0.214	0.034	0.021	0.048	0.033	0.028
Wintering Hills W (3)	0.268	0.034	0.026	0.050	0.028	0.032
Horsethief Canyon (4)	0.182	0.122	0.114	0.124	0.131	0.116
Lousana (5)	0.194	0.146	0.141	0.154	0.155	0.148
Morrin Bridge (6)	0.204	0.125	0.114	0.123	0.133	0.121
North Drumheller (7)	0.212	0.139	0.129	0.146	0.145	0.142
Orkney Lookout (8)	0.176	0.122	0.115	0.129	0.128	0.124
Tolman Bridge (9)	0.200	0.136	0.133	0.142	0.146	0.139
Antler Hill (10)	0.260	0.009	0.005	0.010	0.004	0.007
Bragg Creek Ski Hill (11)	0.264	0.045	0.030	0.046	0.036	0.041
Buck Mtn (12)	0.218	0.009	0.002	0.017	0.007	0.007
Fish Butte (13)	0.216	0.040	0.027	0.044	0.039	0.029
Jumpingpound Ridge (14)	0.271	0.035	0.023	0.044	0.038	0.042
Medicine Hills (15)	0.279	0.011	0.005	0.002	0.009	0.009
Mesa Butte (16)	0.273	0.041	0.022	0.044	0.036	0.023
Powderface Ridge (17)	0.249	0.026	0.017	0.037	0.028	0.011
Shunda Mtn (18)	0.243	0.018	0.014	0.018	0.021	0.012
Wildcat Hills (19)	0.227	0.045	0.035	0.053	0.043	0.037
Enilda Lookout (20)	0.271	0.004	0.009	0.015	0.011	0.017
Goose Mtn (21)	0.245	0.002	-0.005	0.007	0.003	0.005
House Mtn (22)	0.239	0.002	-0.003	0.020	0.000	0.005
Marten Mtn (23)	0.271	0.022	0.014	0.021	0.029	0.019
Pushwaskau Lookout (24)	0.243	0.004	0.000	0.022	0.006	0.007
Sweathouse Lookout (25)	0.253	-0.002	0.001	0.016	0.002	0.009
Whitecourt Mtn (26)	0.266	0.008	-0.009	0.024	0.010	0.009
Beatton River (27)	-0.006	0.261	0.255	0.283	0.259	0.277
Clayhurst (28)	-0.002	0.193	0.181	0.199	0.196	0.201
Highland Park (29)	0.027	0.174	0.167	0.189	0.184	0.181
Kaufman Hill (30)	0.033	0.195	0.187	0.195	0.201	0.192
Lynx Ridge (31)	0.052	0.203	0.197	0.220	0.201	0.214
Shaftsbury Trail (32)	0.070	0.249	0.248	0.260	0.257	0.263
Taylor (33)		0.262	0.262	0.279	0.265	0.275
Bear Mtn (34)	0.280		0.003	0.013	0.001	0.001
Bullhead Mtn (35)	0.277	**0.003**		0.019	0.003	0.005
Kleskun Hills (36)	0.292	**0.013**	**0.020**		0.020	0.004
Saskatoon Mtn (37)	0.282	**0.003**	**0.001**	**0.024**		0.010
White Mtn (38)	0.297	**0.004**	**0.005**	**0.001**	**0.010**	

Appendix 4.3 Tests for recent populations bottlenecks and expansions. Wilcoxon sign rank test *p* values show heterozygosity deficit (population expansion) or heterozygosity excess (population bottleneck) for the strict stepwise mutation model (SMM) and two-phase model (TPM). Qualitative support for recent population bottlenecks is indicated by the mode-shift method. Bolded values show significant *p* values or mode-shifts. Parenthetical codes after locality names indicate the region of each sample, and ".m" or ".z" indicate a separate species designation where regions are partitioned. RDR: Red Deer River region; FH: Foothills; SH: Swan Hills; PR: Peace River region.

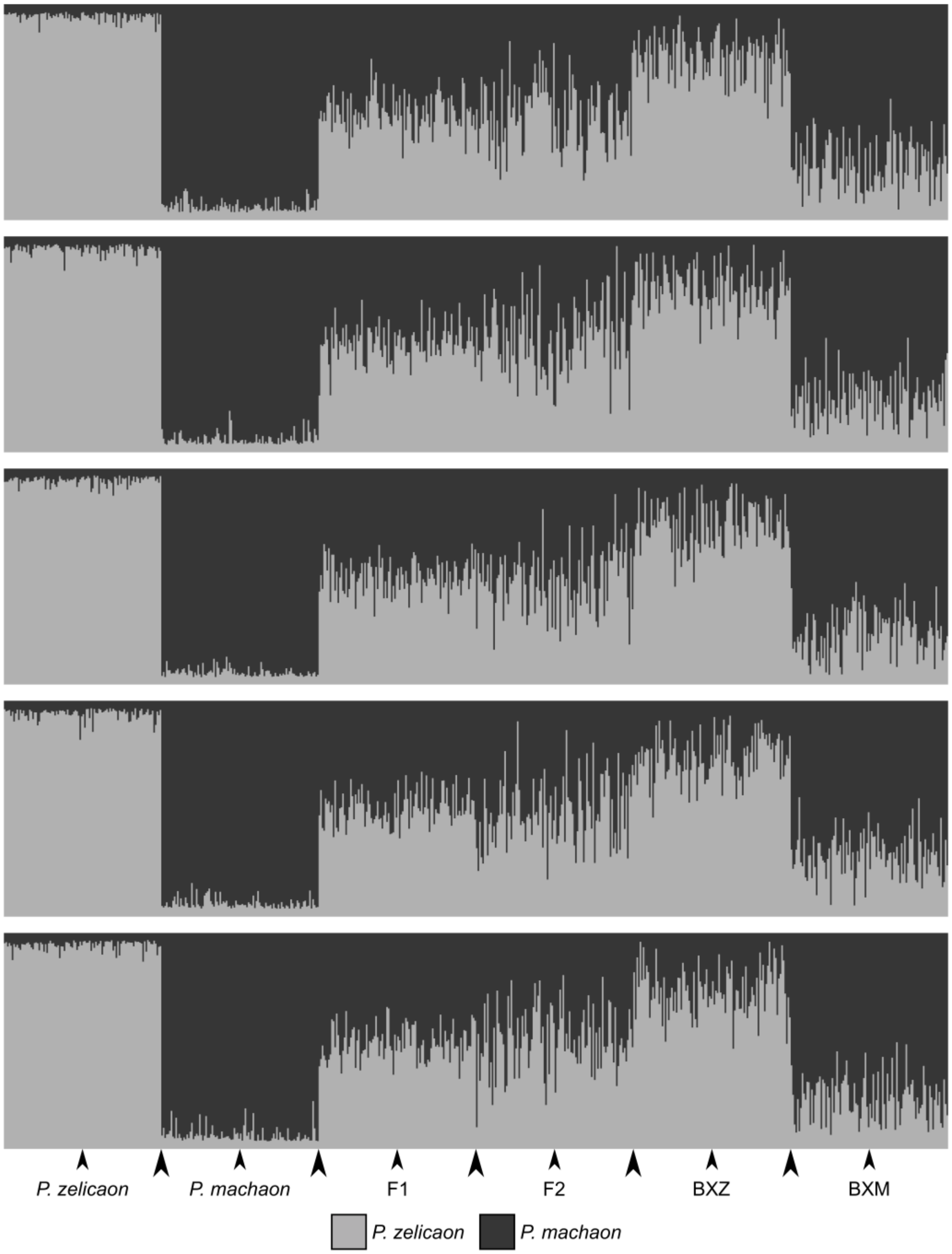
	Heterozygosity deficit		Heterozygosity excess		
	SMM	TPM	SMM	TPM	Mode-shift
Hand Hills (RDR.z)	0.5772	0.8125	0.4609	0.2158	No
Wintering Hills E (RDR.z)	**0.0093**	**0.0161**	0.9932	0.9878	No
Wintering Hills W (RDR.z)	**0.0024**	**0.0093**	0.9985	0.9932	No
Horsethief Canyon (RDR.m)	**0.0024**	**0.0034**	0.9985	0.9976	No
Lousana (RDR.m)	**0.0049**	**0.0068**	0.9971	0.9951	No
Morrin Bridge (RDR.m)	**0.0093**	**0.0093**	0.9932	0.9932	No
North Drumheller (RDR.m)	**0.0005**	**0.0010**	1.0000	0.9995	No
Orkney Lookout (RDR.m)	**0.0020**	**0.0068**	0.9990	0.9951	No
Tolman Bridge (RDR.m)	**0.0024**	**0.0049**	0.9985	0.9966	No
Antler Hill (FH)	**0.0024**	**0.0034**	0.9985	0.9976	No
Bragg Creek Ski Hill (FH)	0.1611	0.1875	0.8623	0.8389	No
Buck Mtn (FH)	**0.0005**	**0.0024**	1.0000	0.9985	No
Fish Butte (FH)	**0.0068**	**0.0420**	0.9951	0.9839	No
Jumpingpound Ridge (FH)	0.8838	0.8838	0.1377	0.1377	**Yes**
Medicine Hills (FH)	0.3477	0.4609	0.6875	0.5772	**Yes**
Mesa Butte (FH)	0.8125	0.8623	0.2158	0.1611	**Yes**
Powderface Ridge (FH)	0.3477	0.5000	0.6875	0.5391	No
Shunda Mtn (FH)	0.4229	0.5391	0.6152	0.5000	No
Wildcat Hills (FH)	0.1611	0.2783	0.8623	0.7539	No
Enilda Lookout (SH)	**0.0122**	0.0654	0.9907	0.9473	No
Goose Mtn (SH)	**0.0024**	**0.0049**	0.9985	0.9966	No
House Mtn (SH)	0.3477	0.4229	0.6875	0.6152	No
Marten Mtn (SH)	0.0801	0.1162	0.9346	0.9033	No
Pushwaskau Lookout (SH)	0.0654	0.1162	0.9473	0.9033	No
Sweathouse Lookout (SH)	0.1875	0.3477	0.8389	0.6875	No
Whitecourt Mtn (SH)	0.6523	0.8389	0.3848	0.1875	No
Beatton River (PR.m)	0.4219	0.4219	0.6289	0.6289	No
Clayhurst Ferry (PR.m)	0.7148	0.7871	0.3262	0.2481	**Yes**
Highland Park (PR.m)	**0.0420**	**0.0420**	0.9839	0.9839	No
Kaufman Hill (PR.m)	**0.0068**	**0.0093**	0.9951	0.9932	No
Lynx Ridge (PR.m)	**0.0024**	**0.0049**	0.9985	0.9966	No
Shaftsbury Trail (PR.m)	0.0645	0.1250	0.9756	0.8984	No
Taylor (PR.m)	0.0820	0.1250	0.9356	0.8984	No

Bear Mtn (PR.z)	**0.0015**	**0.0049**	0.9990	0.9966	No
Bullhead Mtn (PR.z)	0.5000	0.7842	0.5391	0.2461	No
Kleskun Hills (PR.z)	0.3477	0.4609	0.6875	0.5772	No
Saskatoon Mtn (PR.z)	0.1611	0.3125	0.8623	0.7217	No
White Mtn (PR.z)	0.3477	0.3848	0.6875	0.6523	No

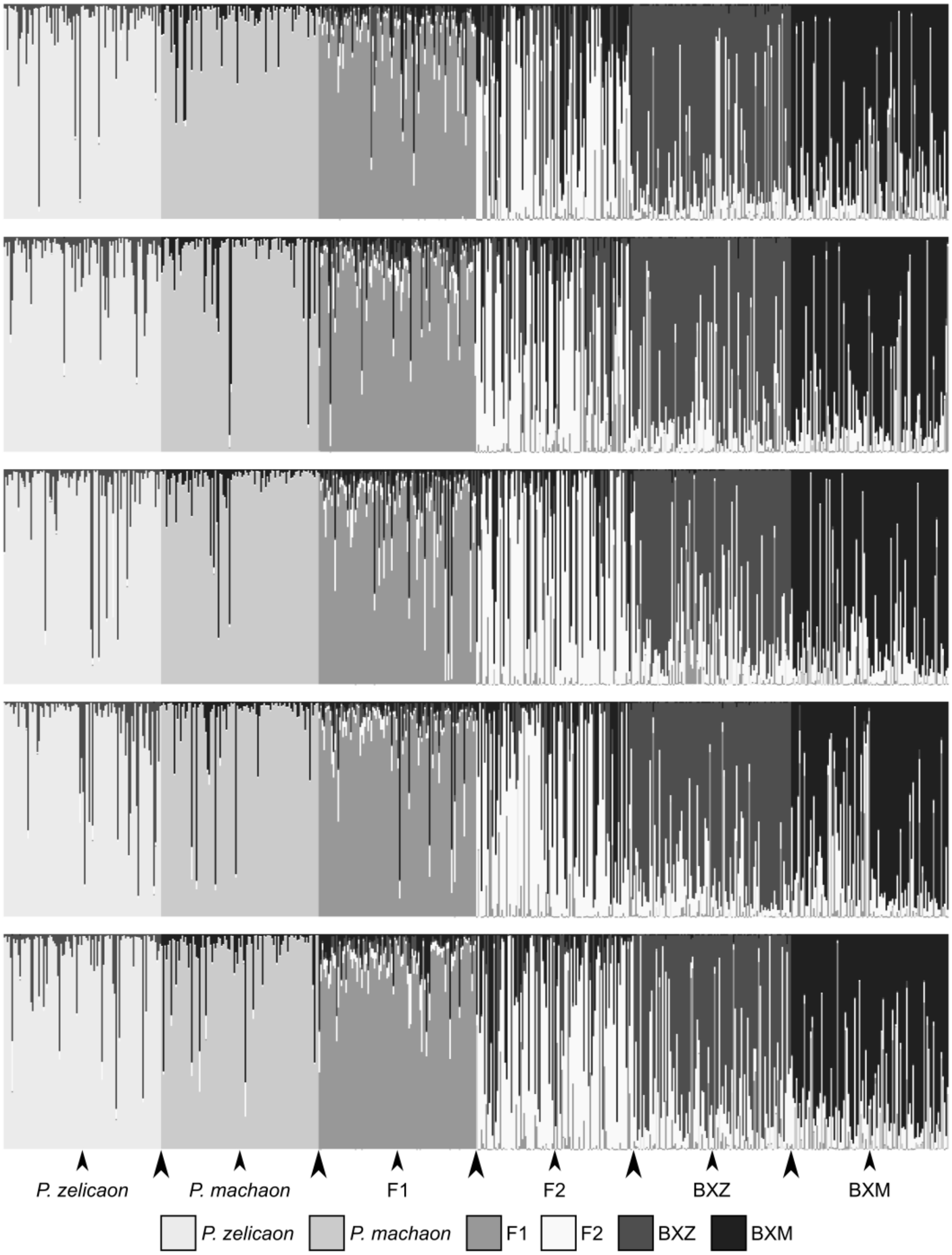
Appendix 4.4 Tests for null alleles, using MICRO-CHECKER (MC) and FreeNA for locality samples, combined regional samples, and species. Parenthetical codes after locality names indicate the region of each sample, and ".m" or ".z" indicate a separate species designation where regions are partitioned. RDR: Red Deer River region; FH: Foothills; SH: Swan Hills; PR: Peace River region. # denotes the number of loci with null alleles present, estimated by MICRO-CHECKER. Freq. nulls/locus denotes the average number of null alleles per locus per locality/region/species, estimated by both MICRO-CHECKER and FreeNA.

		Freq. nulls/locus	
Locality, region or species	#	MC	FreeNA
Hand Hills (RDR.z)	3	0.06368	0.062926
Wintering Hills E (RDR.z)	6	0.10882	0.094239
Wintering Hills W (RDR.z)	4	0.0841	0.073542
Horsethief Canyon (RDR.m)	3	0.08396	0.081443
Lousana (RDR.m)	4	0.08109	0.067744
Morrin Bridge (RDR.m)	4	0.09448	0.092042
North Drumheller (RDR.m)	2	0.07868	0.072813
Orkney Lookout (RDR.m)	4	0.06884	0.092923
Tolman Bridge (RDR.m)	4	0.05234	0.056616
Antler Hill (FH)	3	0.03222	0.059151
Bragg Creek Ski Hill (FH)	4	0.13926	0.123197
Buck Mtn (FH)	8	0.10555	0.099296
Fish Butte (FH)	6	0.09451	0.091949
Jumpingpound Ridge (FH)	2	0.0883	0.09862
Medicine Hills (FH)	4	0.09922	0.128533
Mesa Butte (FH)	2	0.06684	0.084863
Powderface Ridge (FH)	1	0.02672	0.048225
Shunda Mtn (FH)	4	0.08546	0.092279
Wildcat Hills (FH)	1	0.02365	0.044665
Enilda Lookout (SH)	5	0.11056	0.105788
Goose Mtn (SH)	6	0.09436	0.087458
House Mtn (SH)	4	0.08167	0.09325
Marten Mtn (SH)	3	0.04459	0.050586
Pushwaskau Lookout (SH)	4	0.08197	0.080679
Sweathouse Lookout (SH)	3	0.09529	0.087497
Whitecourt Mtn (SH)	2	0.05133	0.058939
Beatton River (PR.m)	2	0.03227	0.057621
Clayhurst Ferry (PR.m)	2	-0.02816	0.058141
Highland Park (PR.m)	3	0.10849	0.103375
Kaufman Hill (PR.m)	4	0.08278	0.07117
Lynx Ridge (PR.m)	3	0.08742	0.084807
Shaftsbury Trail (PR.m)	2	0.01465	0.071963
Taylor (PR.m)	3	0.06933	0.087487
Bear Mtn (PR.z)	5	0.08893	0.08123
Bullhead Mtn (PR.z)	4	0.0771	0.082507

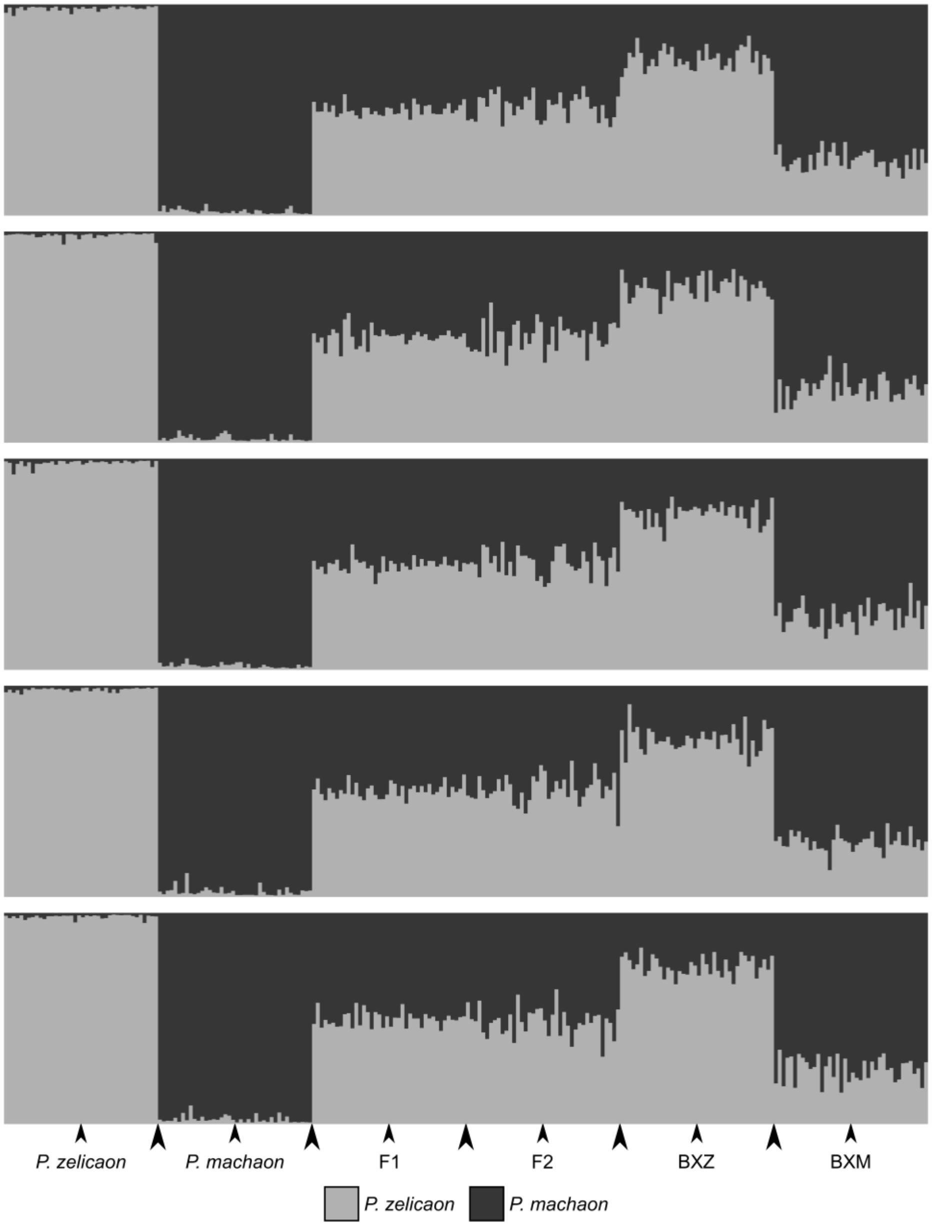
Kleskun Hills (PR.z)	4	0.1046	0.094535
Saskatoon Mtn (PR.z)	5	0.06539	0.077921
White Mtn (PR.z)	6	0.13956	0.133315
all RDR *P. zelicaon* (RD.z)	7	0.08661	0.082443
all RDR *P. machaon* (RD.m)	7	0.0995	0.090089
all Foothills (FH)	9	0.10767	0.101922
all Swan Hills (SH)	8	0.09751	0.092892
all PR *P. machaon* (PR.m)	6	0.09105	0.090632
all PR *P. zelicaon* (PR.z)	8	0.10579	0.097547
All *P. zelicaon*	10	0.10708	0.09996
All *P. machaon*	9	0.13504	0.119677



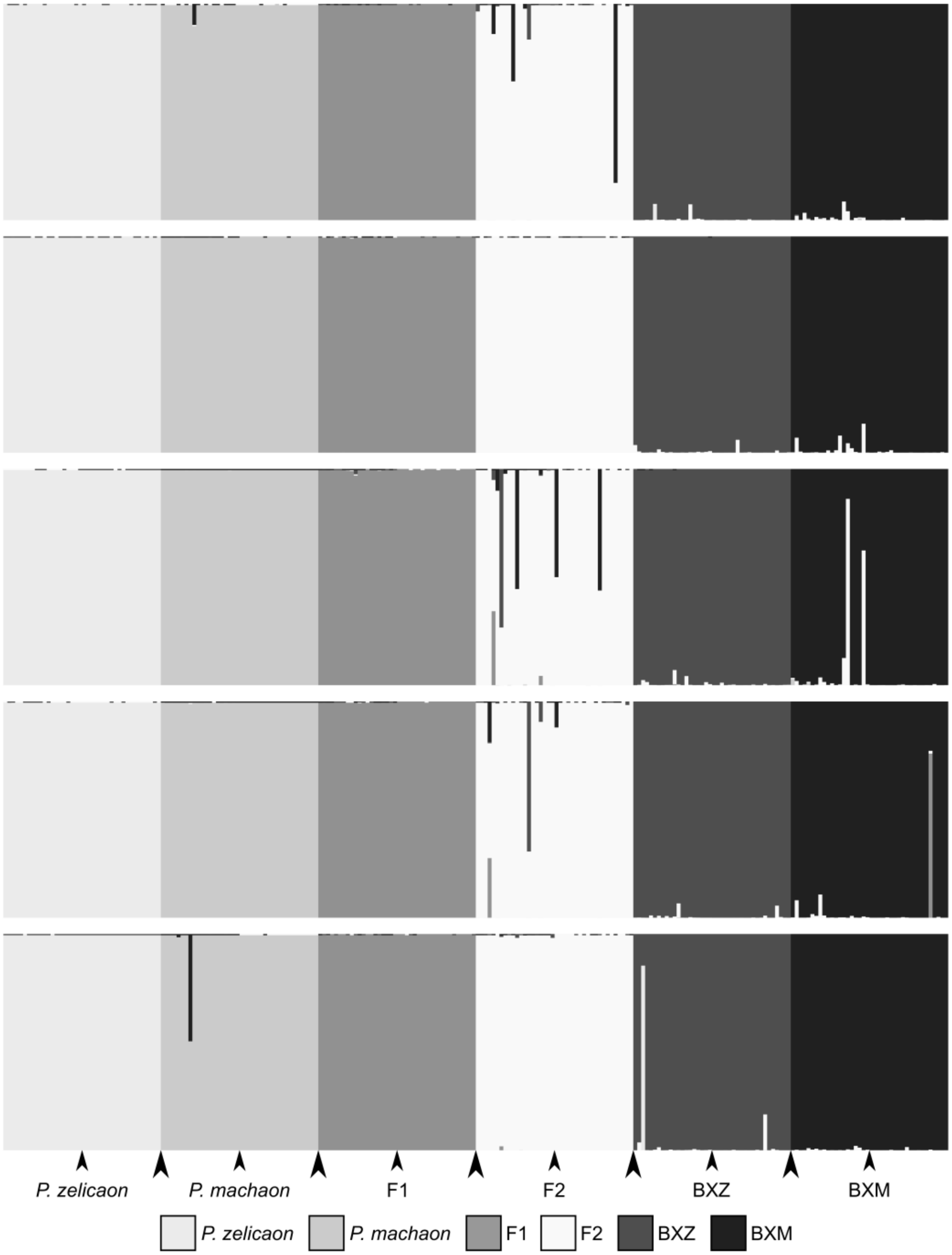
Appendix 4.5 Replicate STRUCTURE runs for simulated microsatellite data. Large arrows below barplots denote simulated parental and hybrid classes, and small arrows indicate division between individuals simulated from the Red Deer River valley (left of small arrow within each class) and Peace River valley (right of small arrow within each class).



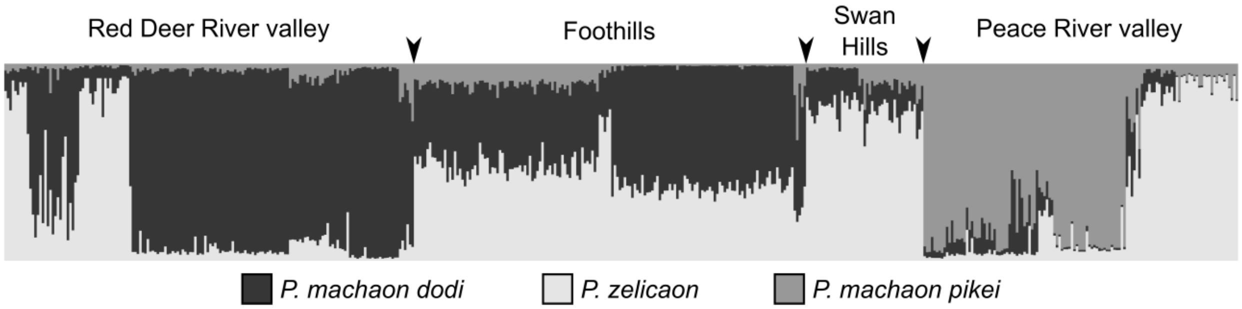
Appendix 4.6 Replicate NewHybrids runs for simulated microsatellite data. Large arrows below barplots denote simulated parental and hybrid classes, and small arrows indicate division between individuals simulated from the Red Deer River valley (left of small arrow within each class) and Peace River valley (right of small arrow within each class).



Appendix 4.7 Replicate STRUCTURE runs for simulated SNP data. Large arrows below barplots denote simulated parental and hybrid classes, and small arrows indicate division between individuals simulated from the Red Deer River valley (left of small arrow within each class) and Peace River valley (right of small arrow within each class).



Appendix 4.8 Replicate NewHybrids runs for simulated SNP data. Large arrows below barplots denote simulated parental and hybrid classes, and small arrows indicate division between individuals simulated from the Red Deer River valley (left of small arrow within each class) and Peace River valley (right of small arrow within each class).



Appendix 4.9 *k* = 3 STRUCTURE results for allozyme dataset from Sperling (1987). Dataset consists of 494 individuals genotyped for 10 allozyme loci. Some individuals are from the same localities sampled in the current study; others are from populations within the same geographic regions.

Appendix 5.1 (next page) Resistance schemes for environmental and landscape variables, and results from Mantel tests for resistance scheme optimization. Resistance values range from 1 (low resistance) to 100 (high resistance). Mantel test results include an R^2-value and a P-value in parentheses. Significant Mantel tests are italicized and the resistance scheme with the highest correlation for each genetic measure is bolded. Missing data represent null surfaces. MSATs: microsatellite dataset; COI: *Cytochrome oxidase subunit I* dataset; Q: *Q*-distance (absolute distance matrix created from STRUCTURE results population averages); FH: genetic measures for populations in the Foothills region; MAT: mean annual temperature; MAP: mean annual precipitation; EMT: extreme minimum temperature over 30 year period.

Land cover feature	res1	res2	res3	res4	res5
water	100	100	100	100	100
snow/ice	100	100	100	100	100
developed	100	75	75	50	75
coniferous	75	50	75	25	50
coniferous dense	75	50	75	25	50
coniferous sparse	75	50	75	25	25
broadleaf	75	50	75	25	50
broadleaf open	75	50	75	25	50
broadleaf sparse	75	50	75	25	25
mixedwood	75	50	75	25	50
mixedwood dense	75	50	75	25	50
mixedwood open	75	15	1	10	10
mixedwood sparse	75	50	75	25	25
shrubland	25	15	1	10	10
shrub tall	25	15	1	10	10
shrub low	25	15	1	10	10
wetland treed	50	50	1	25	25
wetland shrub	25	15	1	10	10
unclassified	10	10	1	1	1
cloud	10	10	1	1	1
non-vegetated	50	15	1	1	1
rock	50	15	1	1	1
exposed	50	15	1	1	1
bryoids	25	15	1	1	1
wetland	1	1	1	1	1
wetland herb	1	1	1	1	1
herbs	1	1	1	1	1
grassland	1	1	1	1	1
agriculture	1	1	1	1	1
agri-cropland	1	1	1	1	1
agri-pasture	1	1	1	1	1
MSATs Nei's D	-0.1465 (0.1440)	-0.1487 (0.1240)	-0.1144 (0.2400)	**-0.1605 (0.1070)**	-0.1454 (0.1600)
MSATs G''_{ST}	-0.0670 (0.3060)	-0.0665 (0.2720)	**-0.0473 (0.3610)**	-0.0922 (0.2070)	-0.0835 (0.2140)
MSATs Q	0.1479 (0.1470)	0.1354 (0.1580)	0.2489 (0.0650)	**0.1015 (0.2080)**	0.1828 (0.1030)
COI Jost's D	*0.3372 (0.0140)*	*0.3472 (0.0065)*	***0.2977 (0.0395)***	*0.2434 (0.0372)*	*0.2814 (0.0345)*
FH MSATs Nei's D	-0.0095 (0.5044)	0.0185 (0.4453)	0.0509 (0.3416)	**0.0760 (0.3240)**	0.0522 (0.3706)
FH MSATs G''_{ST}	-0.0279 (0.4407)	-0.0189 (0.4576)	**0.0318 (0.3978)**	0.0165 (0.4499)	0.0064 (0.4696)
FH MSATs Q	0.1442 (0.1793)	0.1771 (0.1238)	**0.1950 (0.1152)**	0.1839 (0.1081)	0.1916 (0.1068)
FH COI Jost's D	0.0571 (0.3598)	-0.0988 (0.2635)	*-0.2814 (0.0335)*	-0.2324 (0.0575)	***-0.2824 (0.0353)***

MAT value	res1	res2	res3	res4	res5
-5.56 to -4.62	80	60	40	20	1
-4.61 to -3.69	70	50	30	10	1
-3.68 to -2.75	60	40	20	1	10
-2.74 to -1.82	50	30	10	1	20
-1.81 to -0.88	40	20	1	10	30
-0.87 to 0.05	30	10	1	20	40
0.06 to 0.99	20	1	10	30	50
1.00 to 1.93	10	1	20	40	60
1.94 to 2.86	1	10	30	50	70
2.87 to 3.80	1	20	40	60	80
MSATs Nei's D	*-0.2907 (0.0230)*	*0.2932 (0.0160)*	*0.2413 (0.0250)*	0.0250 (0.1330)	0.0346 (0.2750)
MSATs G''_{ST}	-0.1537 (0.0970)	0.1495 (0.0740)	0.1367 (0.0770)	0.0757 (0.1750)	0.0264 (0.3180)
MSATs Q	-0.0762 (0.3180)	0.0589 (0.3070)	**0.1779 (0.0520)**	*0.1395 (0.0970)*	*0.0867 (0.1520)*
COI Jost's D	0.1340 (0.1777)	-0.1465 (0.0985)	0.0034 (0.5276)	0.0499 (0.2500)	0.0575 (0.1637)
FH MSATs Nei's D	-0.1051 (0.3112)	0.1454 (0.2091)	0.2196 (0.1028)	*0.2622 (0.0317)*	***0.2722 (0.0113)***
FH MSATs G''_{ST}	-0.1123 (0.2534)	0.1587 (0.1471)	0.2239 (0.0567)	*0.2561 (0.0179)*	***0.2669 (0.0097)***
FH MSATs Q	**0.2112 (0.0932)**	-0.1165 (0.2144)	-0.1674 (0.1070)	-0.1280 (0.1256)	-0.0949 (0.1815)
FH COI Jost's D	0.0729 (0.3210)	***0.2868 (0.0211)***	0.1863 (0.1132)	*0.2516 (0.0215)*	*0.2680 (0.0128)*

MAT value	res6	res7	res8	res9	res10
-5.56 to -4.62	100	100	100	50	100
-4.61 to -3.69	75	75	50	25	75
-3.68 to -2.75	50	50	1	1	50
-2.74 to -1.82	50	25	1	1	25
-1.81 to -0.88	25	1	1	1	1
-0.87 to 0.05	25	1	1	1	1
0.06 to 0.99	1	1	1	1	1
1.00 to 1.93	1	1	1	1	1
1.94 to 2.86	1	1	1	25	25
2.87 to 3.80	1	1	1	50	50
MSATs Nei's D			*0.3940 (0.0060)*	***0.3940 (0.0010)***	*0.3859 (0.0070)*
MSATs G''_{ST}			***0.2073 (0.0370)***	*0.2073 (0.0380)*	*0.2023 (0.0350)*
MSATs Q			0.0939 (0.2430)	0.0939 (0.2450)	0.0890 (0.2600)
COI Jost's D			**-0.1714 (0.0817)**	-0.1714 (0.0823)	-0.1713 (0.0837)
FH MSATs Nei's D			0.0863 (0.3261)	0.0863 (0.3320)	0.0893 (0.3274)
FH MSATs G''_{ST}			0.0969 (0.2780)	0.0969 (0.2730)	0.1003 (0.2730)
FH MSATs Q			-0.1183 (0.2274)	-0.1183 (0.2151)	-0.1178 (0.2238)
FH COI Jost's D			0.2130 (0.0974)	0.2130 (0.0992)	0.2165 (0.0923)

MAP value	res1	res2	res3	res4	res5
293 to 533	80	60	40	20	1
534 to 773	70	50	30	10	1
774 to 1013	60	40	20	1	10
1014 to 1253	50	30	10	1	20
1254 to 1494	40	20	1	10	30
1495 to 1734	30	10	1	20	40
1735 to 1974	20	1	10	30	50
1975 to 2214	10	1	20	40	60
2215 to 2454	1	10	30	50	70
2455 to 2695	1	20	40	60	80
MSATs Nei's D	-0.0310 (0.3920)	-0.0035 (0.5300)	0.0558 (0.2700)	0.1617 (0.1130)	
MSATs G’’$_{ST}$	-0.0079 (0.4830)	0.0094 (0.4400)	0.0452 (0.2990)	0.0932 (0.1770)	
MSATs Q	0.0793 (0.1360)	0.1126 (0.1170)	0.1803 (0.0680)	*0.2803 (0.0140)*	
COI Jost's D	0.0885 (0.0904)	0.0998 (0.0907)	0.1164 (0.0978)	0.1180 (0.1671)	
FH MSATs Nei's D	*0.2725 (0.0062)*	*0.2741 (0.0053)*	*0.2749 (0.0085)*	0.2487 (0.0658)	
FH MSATs G’’$_{ST}$	*0.2838 (0.0056)*	*0.2930 (0.0038)*	*0.3108 (0.0034)*	***0.3497 (0.0036)***	
FH MSATs Q	-0.0642 (0.2793)	-0.0714 (0.2527)	-0.0920 (0.1774)	-0.1377 (0.1432)	
FH COI Jost's D	*0.2806 (0.0080)*	***0.2808 (0.0078)***	*0.2756 (0.0087)*	0.2089 (0.0718)	

MAP value	res6	res7	res8	res9	res10
293 to 533	100	100	100	100	75
534 to 773	100	50	50	75	50
774 to 1013	50	1	1	50	25
1014 to 1253	25	1	1	25	1
1254 to 1494	1	1	1	10	1
1495 to 1734	1	25	1	1	1
1735 to 1974	1	50	1	1	1
1975 to 2214	25	100	25	1	1
2215 to 2454	50	100	50	1	1
2455 to 2695	100	100	100	1	1
MSATs Nei's D	0.0200 (0.3660)	0.2133 (0.0730)	**0.2170 (0.0610)**	0.0822 (0.2360)	0.1271 (0.1670)
MSATs G’’$_{ST}$	0.0168 (0.3930)	0.1162 (0.1550)	**0.1186 (0.1430)**	0.0540 (0.2650)	0.0841 (0.1990)
MSATs Q	0.1189 (0.1380)	*0.3127 (0.0130)*	***0.3149 (0.0100)***	0.1983 (0.0540)	*0.2519 (0.0250)*
COI Jost's D	0.1035 (0.1023)	0.1125 (0.1944)	0.1129 (0.2022)	0.1173 (0.1077)	**0.1249 (0.1255)**
FH MSATs Nei's D	***0.2839 (0.0082)***	0.2329 (0.0935)	0.2329 (0.0908)	*0.2835 (0.0094)*	*0.2665 (0.0227)*
FH MSATs G’’$_{ST}$	*0.2736 (0.0077)*	*0.3357 (0.0049)*	*0.3357 (0.0065)*	*0.3215 (0.0023)*	*0.3264 (0.0029)*
FH MSATs Q	-0.0906 (0.1985)	-0.1443 (0.1490)	**-0.1444 (0.1500)**	-0.1112 (0.1463)	-0.1172 (0.1520)
FH COI Jost's D	*0.2172 (0.0304)*	0.1699 (0.1457)	0.1698 (0.1408)	*0.2317 (0.0215)*	*0.2584 (0.0156)*

EMT value	res1	res2	res3	res4	res5
-50.7 to -49.8	80	60	40	20	1
-49.7 to -48.8	70	50	30	10	1
-48.7 to -47.8	60	40	20	1	10
-47.7 to -46.8	50	30	10	1	20
-46.7 to -45.8	40	20	1	10	30
-45.7 to -44.8	30	10	1	20	40
-44.7 to -43.8	20	1	10	30	50
-43.7 to -42.8	10	1	20	40	60
-42.7 to -41.8	1	10	30	50	70
-41.7 to -40.8	1	20	40	60	80
MSATs Nei's D	-0.1089 (0.1850)	***-0.2489 (0.0250)***	-0.1330 (0.1750)	0.0705 (0.2810)	-0.0032 (0.5210)
MSATs G''_{ST}	-0.1097 (0.0990)	***-0.2193 (0.0120)***	-0.0148 (0.4650)	0.1085 (0.1420)	0.0418 (0.2850)
MSATs Q	-0.0407 (0.3640)	-0.1240 (0.2000)	0.0562 (0.3150)	0.1196 (0.1650)	0.0772 (0.2080)
COI Jost's D	0.1694 (0.0572)	0.1546 (0.1371)	-0.1709 (0.0787)	-0.0786 (0.2425)	-0.0068 (0.5189)
FH MSATs Nei's D	0.0904 (0.2975)	0.0800 (0.3023)	0.2440 (0.0894)	***0.3027 (0.0073)***	*0.3011 (0.0032)*
FH MSATs G''_{ST}	0.1419 (0.1546)	0.0273 (0.4125)	0.1420 (0.1683)	*0.2437 (0.0183)*	***0.2659 (0.0079)***
FH MSATs Q	0.0303 (0.3603)	**0.2191 (0.1000)**	-0.0300 (0.4322)	-0.1039 (0.1676)	-0.0774 (0.2203)
FH COI Jost's D	*0.2463 (0.0407)*	0.0431 (0.3660)	-0.0345 (0.4170)	*0.2127 (0.0372)*	*0.2526 (0.0164)*

EMT value	res6	res7	res8	res9	res10
-50.7 to -49.8	100	100	100	50	100
-49.7 to -48.8	75	75	50	25	75
-48.7 to -47.8	50	50	1	1	50
-47.7 to -46.8	50	25	1	1	25
-46.7 to -45.8	25	1	1	1	1
-45.7 to -44.8	25	1	1	1	1
-44.7 to -43.8	1	1	1	1	1
-43.7 to -42.8	1	1	1	1	1
-42.7 to -41.8	1	1	1	25	25
-41.7 to -40.8	1	1	1	50	50
MSATs Nei's D	-0.2138 (0.0820)	*-0.2322 (0.0010)*			*-0.2364 (<0.0001)*
MSATs G''_{ST}	*-0.1855 (0.0440)*	*-0.1891 (0.0220)*			*-0.1901 (0.0180)*
MSATs Q	**-0.1518 (0.1350)**	-0.0737 (0.4270)			-0.0739 (0.4320)
COI Jost's D	**0.1956 (0.0939)**	-0.0264 (0.5152)			-0.0266 (0.5074)
FH MSATs Nei's D	*0.2623 (0.0064)*				
FH MSATs G''_{ST}	*0.2569 (0.0086)*				
FH MSATs Q	-0.0387 (0.4112)				
FH COI Jost's D	***0.2705 (0.0103)***				

Slope value	res1	res2	res3	res4	res5
0 to 2	70	50	30	10	1
2 to 5	60	40	20	1	1
5 to 10	50	30	10	1	10
10 to 20	40	20	1	10	20
20 to 30	30	10	1	20	30
30 to 40	20	1	10	30	40
40 to 50	10	1	20	40	50
50 to 60	1	10	30	50	60
60 to 70	1	20	40	60	70
70 to 101	50	50	50	70	80
MSATs Nei's D	0.0116 (0.4150)	0.0906 (0.2000)	*0.2244 (0.0290)*	0.1388 (0.1250)	-0.1598 (0.1370)
MSATs G''ST	0.0044 (0.4600)	0.0423 (0.3120)	0.0958 (0.2030)	0.0787 (0.2090)	-0.0560 (0.3200)
MSATs Q	*0.0482 (0.2370)*	**0.0786 (0.2220)**	0.0760 (0.2800)	-0.0440 (0.3870)	-0.0222 (0.5000)
COI Jost's D	0.0738 (0.1362)	**0.0742 (0.1978)**	0.0287 (0.4621)	0.0027 (0.4434)	0.0222 (0.3413)
FH MSATs Nei's D	***0.2280 (0.0376)***	0.1921 (0.0998)	0.0891 (0.3256)	0.1199 (0.2281)	0.1364 (0.2414)
FH MSATs G''ST	***0.2299 (0.0238)***	0.1987 (0.0649)	0.1111 (0.2452)	0.1576 (0.1431)	0.0846 (0.2994)
FH MSATs Q	-0.0860 (0.2355)	-0.1075 (0.1905)	-0.1462 (0.1567)	-0.1094 (0.2088)	0.1545 (0.8326)
FH COI Jost's D	***0.2894 (0.0090)***	*0.2826 (0.0125)*	0.2312 (0.0728)	0.1993 (0.0906)	-0.0757 (0.3361)

Slope value	res6	res7	res8	res9	res10
0 to 2	50	50	50	25	10
2 to 5	25	1	25	1	1
5 to 10	1	1	10	1	1
10 to 20	1	1	1	1	1
20 to 30	1	1	1	1	1
30 to 40	1	25	1	1	1
40 to 50	1	25	1	1	1
50 to 60	1	25	1	1	1
60 to 70	25	50	25	1	1
70 to 101	50	100	50	50	10
MSATs Nei's D	***0.3157 (0.0180)***	*0.3061 (0.0360)*	*0.2643 (0.0350)*	*0.2993 (0.0350)*	*0.2543 (0.0380)*
MSATs G''ST	0.1340 (0.1170)	**0.1395 (0.1260)**	0.1135 (0.1640)	0.1358 (0.1190)	0.1139 (0.1550)
MSATs Q	0.0385 (0.3920)	-0.0595 (0.3810)	0.0522 (0.3410)	-0.0520 (0.3840)	-0.0423 (0.3840)
COI Jost's D	-0.0046 (0.4960)	-0.0592 (0.3771)	0.0020 (0.5283)	-0.0527 (0.3901)	-0.0341 (0.4174)
FH MSATs Nei's D	0.0513 (0.4017)	-0.0001 (0.5558)	0.0679 (0.3663)	0.0331 (0.3910)	0.0864 (0.2971)
FH MSATs G''ST	0.0744 (0.3180)	0.1010 (0.2505)	0.0902 (0.2892)	0.1111 (0.2318)	0.1327 (0.1913)
FH MSATs Q	-0.1589 (0.1373)	**-0.1630 (0.0937)**	-0.1514 (0.1550)	-0.1605 (0.1078)	-0.1479 (0.1335)
FH COI Jost's D	0.2182 (0.1001)	0.1759 (0.1268)	0.2204 (0.0891)	0.2008 (0.1135)	0.2385 (0.0733)

Biography

On May 8, 1987, I was born in Lansing, Michigan as the second child to my parents, Earl and Sandra Dupuis. The first years of my life were spent in the mixed forests and creeks of central and southwest Michigan, and it was there where my passion for biology and natural history was born. Formal education strengthened my passion for these subjects throughout primary and secondary school, as well as my other interests including art and music. With the guidance of many excellent teachers, both formal and informal, my high school career culminated in independent scientific projects in entomology, an art portfolio focusing on sculptural welding, and performing Dragonetti's concerto in A major for double bass, with full orchestral accompaniment.

My education continued in Marquette, Michigan in 2005 where I pursued a bachelor's degree in biology and chemistry at Northern Michigan University. The wilderness of the upper peninsula of Michigan provided a rich backdrop for my biological passion to grow, and I was fortunate enough to start conducting research my first year in the conservation genetics lab of Alec Lindsay (and continued working with Alec during my entire degree). Here I worked on various population genetics-based projects on the common loon, and developed many skills that would transfer to my research as a graduate student. My course work focused on aquatic ecology and entomology, and during the summers of my Bachelors I worked various field jobs, including electroshocking larval lamprey in the Great Lakes region, conducting stream monitoring for the USFS in the western USA, and as a fisheries biologist technician for the National Park Service in Glacier National Park. During these years, I also "traded in" the double bass for the mandolin, which was a little easier to travel with back and forth across the country every summer.

For graduate school, I wanted to combine my passions of entomology and population genetics, and I came to Edmonton and the University of Alberta in 2009 to start my M.Sc. with Felix Sperling. With Felix's guidance, this research gradually expanded into a Ph.D., and another passion developed in my life: swallowtail butterflies.