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Introgression in a water strider hybrid zone

by

Tricia Akemi Abe

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Systematics and Evolution

Department of Biological Sciences

Edmonton, Alberta

Spring, 2002

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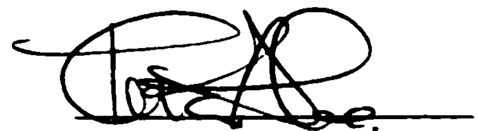

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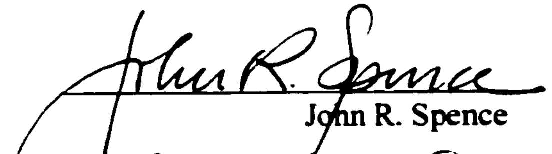
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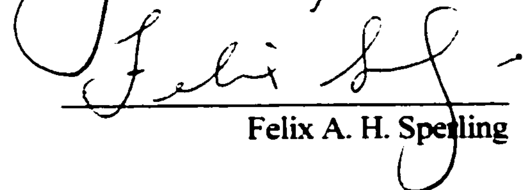
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Colleen C. St. Clair

M. Anne Naeth

January 29, 2002.

For my parents, Doug and Karen,
and my grandparents, Kie and Marie and Aki and Mitzi.

ABSTRACT

This thesis examines the genetic and morphological consequences of hybridization between two species of hybridizing water striders, *Limnoporus notabilis* and *L. dissortis*, in western Canada. In addition, it helps define the geographical boundaries of introgression. An investigation of the availability and suitability of habitats across the hybrid zone suggests that an environmental influence on population density accounts for a patchy population structure. Morphological and genetic markers are developed to identify hybrids in wild populations. Comparisons of introgression patterns for three genetic markers show that species boundaries are selectively permeable, depending on the marker used. The results of this work suggest that interactions between environment and genetics, and genetic incomptabilities, influence patterns of introgression in this hybrid zone. Predicting long-term consequences of natural hybridization on evolution and conservation of species requires knowledge of the effect of hybridization on species, and an understanding of the factors influencing hybrid zone dynamics.

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GENERAL INTRODUCTION

Hybridization between two water strider species, *Limnoporus notabilis* (Drake and Hottes) and *L. dissortis* (Drake and Harris), occurs along the foothills of the Rocky Mountains in Alberta, Canada, as well as in the interior of British Columbia (Spence 1990, Sperling and Spence 1991, Klingenberg et al. 2000). Because many diverse taxa, from deer (Hornbeck and Mahoney 2000) to trees (Raup 1946) hybridize in the foothills, this region probably represents a zone of secondary contact, where species have re-established contact with each other after a period of separation (Remington 1968). In this thesis, *Limnoporus* water striders are used to address questions about the influence of environmental and genetic factors on the extent, direction and geographical limits of hybridization in the foothills. Hybrid zone processes are similar to those involved in the speciation process because both involve barriers to gene exchange. Although studies of hybrid zones are interesting in their own right, and highlight concerns for species conservation (Simberloff 1996), much of the interest in hybrid zones stems from the insights they offer into the speciation process (e.g. Arnold 1997, Howard and Berlocher 1998).

Genetic Processes

Previous studies suggest that genetic incompatabilities, in the form of selection against female hybrids, play a large part in structuring this hybrid zone (Spence 1990, Sperling and Spence 1991, Sperling et al. 1997). Under Barton and Hewitt's (1985) tension zone model of hybrid zone structure, the important determinants of hybrid zone width are strength of selection against hybrids and dispersal capacity of parental species.

For example, relatively weak selection against hybrids results in a broad hybrid zone and strong selection against hybrids leads to a comparatively narrow zone. An assumption of the tension zone model is that relatively low hybrid fitness (Barton and Hewitt 1985) sets limits to the geographical boundaries of hybridization. Because there is no evidence of reduced fitness for *Limnoporus* hybrid males (Spence 1990), factors other than genetic incompatabilities in females must also contribute to shaping the *Limnoporus* hybrid zone.

Ecological processes

Environmental selection may also influence the structure and dynamics of this hybrid zone, if *L. notabilis* and *L. dissortis* are adapted to exploit different habitat characteristics, such as vegetation composition (Spence 1981). The idea that selection for different habitat types occurs along a broad environmental cline, with each species selected for at either end of the cline (Endler 1977), may apply to this hybrid zone. Transitions from forested habitats to those of a dry, prairie landscape, occur from British Columbia to Alberta. If selection acts along this gradient, it may account for an "island" of hybridization in central British Columbia (Spence 1990, Sperling and Spence 1991). An alternative framework for the *Limnoporus* hybrid zone is the mosaic model (Harrison 1986), which predicts non-clinal transitions across a hybrid zone. This model assumes that species have evolved divergent habitat preferences, and that habitat types are scattered across a heterogenous landscape. Therefore, both the characteristics and distribution of habitats are emphasized as important factors influencing the genetic structure of hybrid zones.

Relationships between habitat characteristics and distribution have not been measured in the *Limnoporus* hybrid zone. At first glance, both species occupy similar habitats; shallow, slow-moving or stagnant aquatic habitats with some surface or shoreline vegetation (Spence 1981). However, species preferences for different habitat types might account for the distribution of *L. dissortis* and hybrids in the dry interior of British Columbia, where aquatic habitats appear similar to those of *L. dissortis* in eastern Alberta. Environmental features might also account for the movement of this hybrid zone in some regions (Klingenberg et al. 2000), if habitat characteristics change with time. Presently, the idea that *L. notabilis* and *L. dissortis* occupy different habitat types cannot be ruled out.

Habitat distribution may be an important factor in the *Limnoporus* hybrid zone because dispersal between habitats is frequent and is probably influenced by the distance separating suitable habitats (Spence 2000). *Limnoporus* undergo diapause in terrestrial habitats during winter, colonize temporary aquatic habitats in the spring, and fly between habitats during the summer (Spence and Andersen 1994, Spence 2000). Habitat distribution may account for a patchy pattern of introgression if the distribution of preferred habitat types for each species varies within the hybrid zone. For example, under this hypothesis, in regions where habitats preferred by both species are present, the extent of hybridization will be relatively low when compared to another region where only one habitat type is found (MacCallum et al. 1998). If habitat preferences are similar among species, habitat distribution can increase opportunties for hybridizing when

habitats are relatively close together. Conversely, a physical barrier to dispersal may result from a lack of suitable habitats (Nichols and Hewitt 1986).

Asymmetrical Introgression

Asymmetrical introgression, from *L. dissortis* populations westward into *L. notabilis* populations (Sperling and Spence 1991), must also be accounted for in any explanation of this hybrid zone. Selection on male mating tactics can be an important factor in unidirectional hybridization (Wirtz 1999) and species differences have been suggested in behavioural observations of *L. dissortis* and *L. notabilis* (Spence and Wilcox 1986); smaller bodied *L. dissortis* males are more likely to gain interspecific matings than *L. notabilis* males when they use a "sneaky male" strategy (Spence and Wilcox 1986). Because the success of interspecific matings is rarely confirmed in wild populations, molecular markers can be used to indirectly assess the direction of interspecific matings.

Overview of thesis

In chapter two, I develop one mitochondrial and two nuclear markers to describe patterns of introgression along a transect through the hybrid zone. Distribution patterns of these markers are compared to assess the direction of mating and test if functional male hybrids facilitate gene flow. Because it is maternally inherited, mitochondrial DNA should be a good marker of species boundaries in this hybrid zone if selection acts to limit the dispersal of females and not males (Avise 1994). Demonstrating low fitness in females, which are the homozygous sex in *Limnoporus* (Spence and Maddison 1986), suggests an uncommon exception to Haldane's rule (1922), which predicts higher rates of sterility or inviability in hybrids of the heterogametic sex (Sperling 1993, 1994).

Chapter three examines morphological variation among *L. notabilis*, *L. dissortis* and their hybrids to test assumptions about the degree of variation within parental and hybrid populations (Neff and Smith 1979). Few studies have tested assumptions of hybrid intermediacy using individuals of known parentage. Laboratory-reared specimens of known genotypes were measured at seven traits and compared to indivduals collected from wild populations. I use discriminant functions to assess if *L. notabilis*, *L. dissortis* and their hybrids are morphometrically distinguishable groups. Using morphology to identify hybrids offers a relatively simple basis for identifying areas of hybridization in wild populations.

Chapter four addresses questions about divergent habitat associations among species, and patterns of habitat distribution across the hybrid zone. Habitats with abundant *Limnoporus* populations were sampled along transects, characterized ecologically and described in terms of *Limnoporus* species composition. The mean geographic distances separating *Limnoporus* habitats were estimated and suggest that barriers to dispersal, in the form of low habitat availability, probably contribute to the patchy spatial structure of this hybrid zone. At a smaller scale, habitats were characterized ecologically and described by *Limnoporus* abundance to ask if habitat characteristics influence population density. The results of this study suggest that patterns of interaction between *Limnoporus* species and their hybrids may be informed further by detailed examinations of habitat availability and population density.

Studies that use molecular or morphological markers to document the extent of hybridization must also be considered in light of the historical context of species

interactions. Although the difficulty of separating historical and ecological hypotheses is not easily solved (Endler 1982), hypotheses generated from pattern-oriented studies can be tested and further explored in experiments. The results of this work will help define some exciting avenues for further studies about the maintenance of species integrity in the *Limnoporus* hybrid zone, both from a genetic and an environmental perspective.

REFERENCES

Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York, New York.

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York, New York.

Barton, N.H. and Hewitt, G.M. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113-48.

Endler, J.A. 1977. Geographic Variation, Speciation, and Clines. Princeton University Press, Princeton, New Jersey.

Endler, J. 1982. Problems in distinguishing historical from ecological factors in biogeography. American Zoologist 22: 441-452.

Haldane, J.B.S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12: 101-109.

Harrison, R.G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56: 337-349.

Hornbeck, G.E. and Mahoney, J.M. 2000. Introgressive hybridization of mule deer and white-tailed deer in southwestern Alberta. Wildlife Society Bulletin 28: 1012-1015.

Howard, D.J. and Berlocher, S.H. 1998. Endless Forms: Species and Speciation. Oxford University Press, New York, New York.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. Journal of Evolutionary Biology 13: 756-765.

MacCallum, C.J., Nürnberger, B., Barton, N.H. and Szymura, J.M. 1998. Habitat preference in the *Bombina* hybrid zone in Croatia. Evolution 52: 227-239.

Neff, N.A. and Smith, G.R. 1979. Multivariate analysis of hybrid fishes. Systematic Zoology 28: 176-196.

Nichols, R.A. and Hewitt, G.M. 1986. Population structure and the shape of a chromosomal cline between two races of a *Podisma pedestris* (Orthoptera: Acrididae). Biological Journal of the Linnean Society 29:301-316.

Raup, H.M. 1946. Phytogeographic studies in the Athabasca-Great Slave Lake region, II. Journal of the Arnold Arboretum 27: 1-85.

Remington, C.L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evolutionary Biology 2: 321-428.

Simberloff, D. 1996. Hybridization between native and introduced wildlife species: Importance for conservation. Wildlife Biology 2: 143-150.

Spence, J.R. 1981. Experimental analysis of microhabitat selection in water-striders (Heteroptera: Gerridae). Ecology 62: 1505-1514.

Spence, J.R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Canadian Journal of Zoology 68: 1770-1782.

Spence, J.R. 2000. Seasonal aspects of flight in water striders (Hemiptera: Gerridae). Entomological Science 3: 399-417

Spence, J.R. and Andersen, N.M. 1994. Biology of water striders: Interactions between systematics and ecology. Annual Review of Entomology 39: 101-128

Spence, J.R. and Maddison, D.R. 1986. Chromosomes of two hybridizing species of *Limnoporus* (Heteroptera: Gerridae). Proceedings of the Entomological Society of Washington 88: 502-508.

Spence, J.R. and Wilcox, R.S. 1986. The mating system of two hybridizing species of water striders (Gerridae) II. Alternative tactics of males and females. Behavioural Ecology and Sociobiology 19: 87-95.

Sperling, F.A.H. 1993. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. Heredity 71: 227-233.

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

Sperling, F.A. H. and Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370-1383.

Sperling, F.A.H., Spence, J.R. and Andersen, N.M. 1997. Mitochondrial DNA, allozymes, morphology and hybrid compatability in *Limnoporus* water striders (Heteroptera: Gerridae): Do they all track species phylogenies? Annals of the Entomological Society of America 90: 401-415.

Wirtz, P. 1999. Mother species-father species: unidirectional hybridization in animals with female choice. Animal Behaviour

VARIATION IN PATTERNS OF INTROGRESSION FOR MITOCHONDRIAL AND NUCLEAR MARKERS IN A WATER STRIDER (HEMIPTERA: GERRIDAE: *LIMNOPORUS*) HYBRID ZONE

INTRODUCTION

Under most concepts species do not exchange genes (Mayr 1942, Dobzhansky 1951, Paterson 1993, Templeton 1989), despite demonstrations that natural selection acts against gene flow at some loci more than at others (Wang et al. 1997). However, reconciling species concepts with gene exchange in hybrid zones is not problematic if species diverge over only parts of the genome, while sharing variation in other parts of the genome. Assessing patterns of gene flow across multiple loci in hybrid zones allows us to identify genes that are more likely to contribute to reproductive isolation between species, and those that freely cross species' boundaries. Combined with knowledge of the mode of inheritance, function, or location in the genome, information on patterns of introgression can be used to generate hypotheses about mechanisms maintaining species integrity. Understanding how reproductive isolation arises from complex gene interactions is a fundamental question in evolutionary biology that is increasingly informed by studies of gene exchange in hybrid zones (Harrison 1993, Arnold 1997).

Comparisons of patterns of gene exchange of nuclear versus mitochondrial markers can offer unique insights into the direction of mating in hybrid zones, where interspecific matings are rarely observed directly (Avise 1994). Comparisons of biparentally inherited genes (nuclear) to maternally inherited genes (mtDNA) reveals the maternal parent in hybrids, and distinguishes between reciprocal hybridization (females equally likely to mate with a male from either species) and unidirectional hybridization (females of one

species mate with males of the other species but not vice-versa). The relatively common occurence of unidirectional hybridization (Wirtz 1999), emphasizes the importance of factors such as size differences (Grant and Grant 1997, Karl et al. 1995), sneak (Crapon de Caprona 1986) and forced (Robertson 1983) copulations, ecological and behavioural biases (Lamb and Avise 1986) and discrimination intensity (Thulin et al. 1997, Kaneshiro and Giddings 1987) on interspecific mate selection.

Assessing patterns of variation for mitochondrial and nuclear markers can also shed light on differential dispersal of males and females in hybrid zones (Avise 1994). For example, the heterogametic sex commonly shows higher rates of sterility or inviability in F_1 hybrids, in accordance with Haldane's rule (1922). On this basis, taxa such as butterflies and birds, in which females are the heterogametic sex, are expected to have reduced dispersal capacity in hybrid females. Predictions of a Haldane effect are upheld by a correspondance between species boundaries and mtDNA haplotype distributions in several Lepidoptera species pairs (Sperling 1993, Sperling 1994, Prowell 1998), and in a study of hybridizing bird species showing high mtDNA divergence between species with little differentiation of nuclear markers (Tegelström and Gelter 1990).

Conversely, when male dispersal capacity is reduced, patterns of population structure are more difficult to predict because female gene flow will homogenize the distribution of nuclear and mitochondrial genes (Piertney et al. 2000). For example, mtDNA appears to cross some species' boundaries for taxa such as *Drosophila* species (Aubert and Solignac 1990), with heterogametic males and fertile hybrid females. Although sex-biased gene flow can be predicted from studies of hybrid fitness conducted

in the laboratory, the geographic distribution of mitochondrial and nuclear markers across a hybrid zone can provide compelling evidence of the significance of this process in natural populations.

This study compares the distribution patterns of one mitochondrial and two nuclear markers in a hybrid zone between two species of *Limnoporus* water striders, *L. notabilis* and *L. dissortis*. These *Limnoporus* are well-suited for testing predictions of differential gene exchange because previous work in the laboratory (Spence 1990) and on field populations (Sperling and Spence 1991) demonstrates that hybrid females are strongly selected against. Furthermore, behavioural observations suggest a bias in the direction of mating, with *L. dissortis* males gaining more interspecific matings than *L. notabilis* males (Spence and Wilcox 1986, Spence 1990). Variation in male mating tactics between *Limnoporus* species in the hybrid zone may partially account for asymmetrical introgression of *L. dissortis* alleles into *L. notabilis* populations (Sperling and Spence 1991).

I examine nuclear DNA sequence polymorphisms within 2 genes. Elongation factor 1-alpha (EF1-α) and the first internal transcribed spacer region (ITS 1) provide diagnostic base pair differences that consistently differentiate *L. notabilis* from *L. dissortis*, and enable us to identify hybrids. I also sequenced a portion of a mitochondrial gene, cytochrome oxidase subunit 1 (CO1) that has previously been shown to distinguish between *Limnoporus* species (Sperling et al. 1997).

I expected little, if any, evidence of movement of the *L. dissortis*-like mitochondrial marker across the hybrid zone relative to nuclear loci. Furthermore,

because *L. dissortis* males are more likely to mate with a *L. notabilis* female than the reverse combination, I expected that hybrids should carry *L. notabilis* mtDNA more frequently than *L. dissortis* mtDNA. The use of both mitochondrial and nuclear markers not only helps us understand how selection acts on different parts of the *Limnoporus* genome, but also serves to strengthen evidence from previous studies about selection against female hybrids.

METHODS

Screening for diagnostic sequence differences

Initially, my goal was to determine differences in nucleotide composition that could consistently distinguish between species. One mitochondrial (cytochrome oxidase subunit I: CO1) and 2 nuclear (elongation factor one-alpha: EF1-α and the first internal transcribed spacer unit: ITS 1) genes were surveyed for variation in three to five *Limnoporus* specimens per species, collected from populations that were widely spaced geographically. Collecting localities, shown in Table 2-1, were chosen to sample the range of variation within species. Two specimens from George Lake, Alberta were sequenced at the EF1-α and ITS 1 loci. CO1 sequence data for *L. notabilis* collected from Fernie, British Columbia, are not included because only a portion of the entire sequence length produced clean sequences. For some specimens, genomic DNA had already been extracted during a previous study. Four CO1 sequences were available on GenBank (Accession numbers U83333-U83336) (Sperling et al. 1997) and two EF1-α sequences were available (Damgaard, Chapter 4 of Ph.D. thesis). New *Limnoporus* specimens were collected in

June 1999. DNA was extracted from the thoraces of live-frozen (-70°C) specimens using QIAamp spin columns (Qiagen).

PCR amplification and sequencing

About 819 bp of the 826 bp CO1 segment were amplified and sequenced by the polymerase chain reaction (PCR), using the two end primers, Jerry (C1-J-2183) and Pat (TL2-N-3014) (Simon et al. 1994). If chromatogram signals were weak, sequences were obtained from two overlapping fragments using two internal primers, K741 (C1-N-2578a) (Caterino and Sperling 1999) and Brian VI (C1-J-2495g) 5' CTT CTA CAT TAT GAA CAC TAG G 3'. A 487 bp region of a 527 bp EF1-α gene was amplified by PCR using the primers M2412 (Damgaard et al. 2000) and M52.6 (Cho et al. 1995). 252-259-bp of the ITS-1 gene region were amplifed by PCR using primers ITS5 (B.Crespi, Insect nuclear primer kit, University of British Columbia, 5' GGA AGT AAA AGT CGT AAC AAG G 3') and 1.58S (Marcon et al. 1999). In general, PCR amplifications were performed in 53.25 uL solutions containing 35.75 uL RNase free water, 1 uL dNTP (10mM), 5 uL $MgCl_2$ (25mM), 5 uL Promega PCR buffer containing 15 mM $MgCl_2$, 2 ul each primer at 5mM, 2 uL genomic DNA, and 0.5 uL Taq polymerase (manufactured at the University of Alberta). PCR reactions underwent a "hot start", with Taq polymerase addded at the annealing temperature during the first cycle. Programs for all loci were 35 cycles long and varied only in the annealing temperatures. Each program consisted of an initial 2 minute denaturation at 94°C followed by cycles of 30 seconds at 94°C, 1 minute at the annealing temperature (45°C for CO1, 53°C for EF1-alpha and 55°C for ITS 1) and 2 minutes at

72°C. The final extension time was 5 minutes at 72°C. All PCR reactions were run on a Biometra T-gradient thermocycler.

PCR bands were visualized on a 1.5% agarose gel stained with ethidium bromide. PCR products were cleaned using Qiagen's PCR Purification Kit, and cycle sequenced with Amersham's Dye Terminator Cycle Sequencing Kit in a 15 uL solution: 9uL RNase free water, 1 uL purified PCR product, 1 uL primer, and 4 uL sequencing premix. The sequencing products were filtered through Sephadex-packed columns and dried for approximately 20 minutes on a vaccuum centrifuge. The dried product was resuspended and electrophoresed on an Applied Biosystems International 377 automated sequencer. DNA sequences were confirmed with both sense and anti-sense strands.

DNA sequences were aligned in Sequencher (version 4.1) and translated into amino acid sequences using MacClade (version 4.0). Pairwise distances between sequences were calculated with PAUP (4.0b8 version).

Assaying populations within the hybrid zone

To evaluate population structure through the hybrid zone, 10 adult *Limnoporus* were collected from each of nine populations along a transect along Highway 16 in western Alberta, Canada and from one population at The Malcolm Knapp Research Forest in Maple Ridge, British Columbia, in August 2000 (Figure 2-1 and Table 2-2). Individuals were collected with hand nets from the surface of shallow, lentic water bodies and kept alive on moist paper towels for transport back to the laboratory. All specimens were placed in 1.5 ml. centrifuge tubes, frozen live and stored at -70°C. Protocols for

DNA extraction and PCR amplification follow those used to intially screen specimens for sequence variation.

For a survey of variation within natural populations, only 527 bp of the 826 bp CO1 segment were sequenced. About 527 bp could be unambiguously sequenced using only the end primer Jerry (C1-J-2183) (Simon et al. 1994). Thus, using a portion of the total sequence length saved time and expense, while still supporting assay of variation at eight out of eleven diagnostic CO1 positions. CO1 fragments were sequenced in only in the forward direction; if there were ambiguous sites, the sequence was confirmed by sequencing in the opposite direction. The entire 527 bp EF1-α region was sequenced. DNA sequences for EF1-α and ITS-1 were confirmed in both directions.

Determining nuclear haplotypes

I introduce a method of separating diploid nuclear sequences into 2 haplotypes using sequence data from genes isolated with PCR. Identifying nuclear haplotypes from sequence data is difficult because PCR sequences that are heterozygous at more than one nucleotide position can have many alternative genetic configurations and the true arrangment cannot be resolved on a sequence chromatogram. In a previous study, Cooper and Hewitt (1993) separated heterozygous sequences into haplotypes by distinguishing haplotypes based on peak size differences on a sequencing chromatogram.

For this study, nucleotide positions that consistently showed double peaks when sequenced in both directions were scored as heterozygous. I separated heterozygous nuclear genotypes into haplotypes by subtracting known haplotypes from heterozygote

genotypes. Known nuclear haplotypes were those that appeared to be homozygous at all nucleotides. Thus, the complement to the known haplotype in a heterozygote was determined by deduction. Most haplotypes were in a homozygous state and all haplotypes could be inferred with this method. EF1-α haplotypes were homozygous at all positions for 12/27 haplotypes, heterozygous at just one position for 9/27 haplotypes, heterozygous at two positions for 3/27 haplotypes, and heterozygous at 3 positions for 3/27 haplotypes. For ITS 1, 8/8 haplotypes were homozygous at all positions. There were no more than 3 heterozygous positions within any one sequence. Out of all sequenced haplotypes, only two EF1-α haplotypes were not present as homozygote genotypes.

Phylogenetic reconstruction of haplotypes

Phylogenetic relationships among haplotypes were assessed in a parsimony analysis (PAUP 4.0b8) employing a heuristic search with 20 random stepwise addition sequences and branch swapping with no more than 20 trees saved per replicate. The robustness of all trees was tested with bootstrap values calculated from 500 replicates.

Population differentiation

The proportion of shared haplotypes is a simple measure of similarity between populations. For pair-wise population estimates, the number of times a shared haplotype appeared in both populations was counted . The sum of all shared haplotypes

was divided by the total number of gene copies within each pair of populations (i.e. n=20 for mtDNA, n= 40 for nuclear DNA).

Estimates of within-population nucleotide diversity were calculated for CO1, EF1-α and ITS 1 for each of 10 populations using Arlequin 2.0 software (Schneider et al. 2000) . Nucleotide diversity measures the mean number of differences between all pairs of haplotypes.

The null hypothesis of panmixia was tested using an exact test of differentiation of haplotypes or genotypes among populations, using Arlequin 2.0 software (Schneider et al. 2000). The exact test of population differentiation tests a hypothesis of a random distribution of k haplotypes or genotypes among r populations. All potential states of the contingency table are explored with a Markov chain (10 000 steps). I estimated probabilities that the observed table of frequencies is less likely than the table configuration expected under panmixia. The exact test was performed for EF1-α and ITS 1. It was not possible to test CO1 haplotypes because of a high level of polymorphism (see Results). Although reducing the number of CO1 haplotypes to the major lineages would allow a test of population differentiation, the widespread distribution of haplotype lineages would not be useful for detecting population structure at a finer scale.

Estimating gene flow

A cladistic analysis of gene flow among populations was performed by determining the minimum number of migration events(s) necessary to account for the current distribution of haplotypes among 10 populations (Slatkin and Maddison 1989).

Estimates of gene flow were calculated for CO1 and EF1-α. The cladistic analysis of gene flow could not be applied to the ITS 1 phylogeny because of the unresolved tree toplogy (see Results). When the cladistic method of estimating gene flow is applied to very shallow, multifurcating tree topologies, like that of ITS 1, the resulting value of s can be underestimated (Slatkin and Maddison 1989). Phylogenies were constructed with each OTU represented by an individual, with location coded as a multistate, unordered character (MacClade vers. 4.0) (Maddison and Maddison 1992).

Gene flow was also calculated in Arlequin (version 2.0) using analysis of molecular variance (AMOVA) (Excoffier et al. 1992), which produces Φ-statistics similar to F-statistics. Values of Φ_{ST} were used to calculate M, an estimator of the gene flow parameter Nm (the average number of migrants between populations connected by gene flow) by substituting Φ_{ST} for Fst in the equation $M=Nm=1/4[1/Fst - 1]$ for nuclear genes (Slatkin 1993) and $Nm=1/2[1/Fst - 1]$ for mtDNA (Hudson et al. 1992). Values of $M >= 1$ are generally considered sufficient to overcome the effects of genetic drift and prevent population differentiation.

Measures of disequilibrium

Each haplotype or genotype locus was treated as if it had only *L. dissortis*-like or *L. notabilis*-like alleles. Almost all haplotypes were unmistakably classified as *L. dissortis* or *L. notabilis* in a phylogenetic analysis (see Results), so reducing each locus to a two allele system seemed reasonable for tests of nonrandom genetic associations among species. I tested agreement of single locus genotype frequencies with Hardy-Weinberg

expectations for the EF1-α and ITS-1 markers using a test analogous to Fisher's exact test (Arlequin vers. 2.0) with a modified version of the Markov-chain random walk algorithm described by Guo and Thomson (1992). Linkage disequilibrium between the 2 nuclear genes was estimated in Arlequin (version 2.0) using an exact test of linkage disequilibrium for haplotypic data, assuming that the haplotypic composition of the sample is known (i.e. genotypes could be separated into *L. dissortis*-type or *L. notabilis*-type haplotypes).

Associations between mtDNA and nuclear DNA can be examined for evidence of non-random mating by estimating cytonuclear disequilibrium in populations exhibiting intermediate allele frequencies. Four measures of genotypic disequilibrium (D, D_1, D_2, D_3) can provide insights into processes occurring within the hybrid zone, such as direction of crosses between hybridizing taxa and levels of assortative mating (Asmussen et al. 1987, Arnold 1993) . If *L. dissortis* is characterized by a cytonuclear genotype *DD/d* and the cytonuclear genotype of *L. notabilis* is *NN/n*, the genotypic disequilibrium D_1 measures departure from random association between a cytoplasmic gene *d* with a nuclear genotype *DD* (D_1= freq(*DD/d*) - freq(*DD*)*freq(*d*)). D_2 measures the departure of genotype *DN* with *d* (D_2= freq(*DN/d*) - freq(*DN*)*freq(*d*) under random expectations and is a measure of the direction of mating. D_3 measures disequilibrium between *NN* and *d*, such that D_3=freq(*NN/d*) - freq(*NN*)*freq(*d*). D_1 is positive and D_3 negative when *DD* genotypes carry *d* mtDNA more often than would be expected by chance. The gametic disequilibrium parameter, D measures the departure of gametic frequencies from expectations under random association (D= freq(*D/d*) - freq(*D*) * freq(*d*)). Cytonuclear

disequilibria were tested with both nuclear loci using a program written by C.J. Basten (http://www2.ncsu.edu/ncsu/CIL/stat_genetics/basten.html) that generated p values based on Fisher's exact test (Asmussen and Basten 1994).

Hybrid index scores

Associations between all 3 markers were examined by calculating a hybrid index score. A score of 1 was given for each *L. dissortis* allele possessed by an individual, such that a maximum score of 5 (2 alleles from each of 2 nuclear loci, and 1 mitochondrial allele) was given for a pure *L. dissortis*, and a score of 0 represented a pure *L .notabilis* individual. A hybrid individual would possess an intermediate score.

RESULTS

Screening for diagnostic variation

Approximately 819 bp of the 826 bp CO1 segment were sequenced, corresponding to nucleotide positions 2188 to 3007 in *Drosophila yakuba* (Clary and Wolstenholme 1985). The sequences revealed 11 diagnostic nucleotide positions among 7 individuals collected from geographically distant populations (Table 2-1). Percent sequence divergence between species ranged from 1.59-2.08 while intraspecific divergence ranged from 0.49-0.98 for *L. dissortis* and 0.24-0.49 for *L. notabilis* (Table 2-3a).

In the EF1-α segment I found 2 diagnostic positions out of 487 bp, corresponding to nucleotide positions 2417 to 2904 in *Drosophila melanogaster* (Hovemann et al. 1988, GenBank accession number X06869), in 9 individuals representing 8 geographic locations.

Interspecific divergence ranged from 0.41-0.82 while the range of intraspecific divergence was 0.00-0.41 in *L. dissortis* (Table 2-3b). There were no intraspecific differences in EF1-α within *L. notabilis*.

ITS 1 sequences were diagnostic at 11 nucleotide positions, including a 7-bp insertion/deletion distinguishing 252 bp sequences in *L. dissortis* from 259 bp in *L. notabilis*. Each indel bp position was counted as one difference and interspecific divergence ranged from 1.18-1.68 (Table 2-3c). Neither *L. dissortis* and *L. notabilis* showed any intraspecific variation in ITS 1.

Samples collected in the hybrid zone

CO1

Approximately 527 bp of the 826 bp CO1 segment, corresponding to nucleotide positions 2197 to 2725 in *D. yakuba* and encompassing 8 diagnostic nucleotide positions, were sequenced in 100 individuals representing 10 populations; 58 haplotypes were detected. Table 2-4 summarizes the variable nucleotide positions for all 58 CO1 haplotypes. Nucleotide variation in the form of single base pair substitutions was detected at 55 positions; 8 in the first codon position and 47 in the third codon position. There were 47 synonomous and 9 non-synonomous substitutions (one nucleotide position had both types of substitutions), indicating that most of the variation (83.9%) was neutral.

The average number of CO1 haplotypes per population was 7.5, ranging between 6 and 10. Most haplotypes were found within just one population, although 10 haplotypes were found in at least 2 populations. Population 9 in Vancouver had 5 unique haplotypes, representing 60% of the population and shared haplotypes with populations 7 and 8. At the eastern end of the transect, population 0 had 9 unique haplotypes, representing 90% of that population; haplotype D (10% of the population) was shared with populations 2, 3, 4 and 6. The proportion of shared haplotypes ranged from 0.00 to 0.45, with few populations between sites 0 to 6 sharing haplotypes with sites 7 to 9 (Table 2-5). Average nucleotide diversity within populations ranged from 0.0010 (site 0, Niton Junction) to 0.0118 (site 7, east side of Jasper National Park) (Table 2-6).

Only populations 5 (Hinton) and 7 (Jasper National Park) possessed haplotypes of both mtD (*L. dissortis*) and mtN (*L. notabilis*) lineages, with the mtD lineage representing 80% and 30% of the populations, respectively. The transition between the mtD lineage to the mtN lineage occurs between sites 6 and 8, over a distance of about 170 km (Figure 2-2). Populations 1-4 and 6 were monomorphic for haplotypes from the mtD lineage, and populations 8-9 possessed only haplotypes from the mtN lineage.

Phylogenetic analysis of 58 CO1 haplotypes and including the specimens initially used to screen for variation, rooted with 2 *L. rufoscutellatus* sequences as outgroups, resulted in 200 equally parsimonious trees of length 119. *L. rufoscutellatus* sequences represent individuals collected from Alaska and Finland and are available from GenBank (accession numbers U83337 and U83338, Sperling et al. 1997). The tree had a homoplasy index of 0.437.

A strict consensus tree shows the haplotypes separated into 2 main monophyletic groups (Figure 2-3a). *Limnoporus notabilis* haplotypes formed a monophyletic lineage, representing 90 % of the individuals collected within and west of Jasper National Park (populations 7-9) and 20% of individuals collected in Hinton, Alberta (population 5) (Figure 2-3b). Monophyly is also apparent in the *L. dissortis* lineage, with most haplotypes unresolved in a strict consensus tree. The mtD lineage is associated with populations 0-6, although haplotype B was detected in population 7 (representing 20% of that population). mtN lineage haplotypes formed a monophyletic group in 82% of the bootstrap replicates, and mtD lineage was supported with a bootstrap value of 58%. Low support for the monophyly of the mtD lineage suggests that alternative topologies are possible. Overall, the haplotype phylogeny indicates distinct lineages for each of *L. notabilis* and *L. dissortis*, with mtN and mtD lineages occurring west and east of the Rocky Mountains respectively, and both lineages represented in geographically intermediate populations.

EF1-α

527 bp of the EF1-α gene were sequenced from 99 individuals representing 10 populations. One individual in population 0 was excluded because it produced consistently weak sequence chromatograms that were difficult to interpret. Separation of genotypes into 2 haplotypes revealed 27 haplotypes in total. A total of 17 variable nucleotide positions were found (Table 2-7); 1 in the first codon position and 16 in the third codon position. Most of the variation was neutral, as 16 of the variable positions

(94.1%) were synonomous substitutions. The one non-synonomous substitution was found in a nucleotide position that was diagnostic between species. The uncorrected p-distance among 27 alleles was between .002 and .013 (mean= .006).

Haplotype A, an *L.dissortis* (EF.D) lineage haplotype, was the most common, representing 87/198 (44%) of the total samples and was widely distributed (populations 0-8). The second most common haplotype, R, an *L. notabilis* (EF.N) lineage haplotype, was found in populations 3, 5, 6, 7, 8 and 9 and represented 14% of the total samples. Table 2-5 shows the proportion of shared haplotypes between populations. In general, these results suggest a wide overlap in the distribution of EF.D and EF.N lineage haplotypes, although populations 0, 2 and 4 were all EF.D lineage and population 9 had all EF.N lineage. The main transition between EF.D and EF.N lineages occurs between sites 4 and 8, spanning a distance of about 210 km (Figure 2-2), with steep transition between population 6 and 7. Twelve heterozygotes representing both EF.D and EF.N lineage haplotypes, were found. All but one heterozygote was found in populations 5-8, with the greatest number of heterozygotes in population 7 (n=5). There were no heterozygote deficits within populations, suggesting panmixis (Table 2-8). Average nucleotide diversity within populations ranged from 0.0006 (site 0, Niton Junction) to 0.0034 (site 7, east side of Jasper National Park) (Table 2-6).

The proportion of shared haplotypes was greater than 0.5 for all pairwise comparisons between sites 0 to 6 and for comparisons between site 9 with sites 5, 7 and 8 (Table 2-5). An exact test of differentiation of genotype frequencies was significant over all populations (P=<<0.001). A test of differentiation among all pairs of sample

sites showed significant differences (p<.05) in 29 out of 45 comparisons (Table 2-5). Populations located on the western side of the transect (sites 7, 8 and 9) were signficantly differentiated from all sites between 1 and 6, with one exception. In general, sites 0 to 4 were not significantly differentiated from each other.

Two haplotypes merit special attention because they do not fit neatly into *L. dissortis* or *L. notabilis* classifications based on the substitutions at 2 diagnostic positions. Haplotype FF detected in population 9 and haplotype W in population 1 both possessed a *dissortis*-like substitution at the first diagnostic nucleotide position and a *notabilis*-like substitution at the second diagnostic position.

Phylogenetic analysis of 27 EF1-α haplotypes and including the specimens intially used to screen for variation, rooted with *L. rufoscutellatus*, resulted in 380 equally parsimonious trees of length 39 (Figure 2-4). The *L. rufoscutellatus* sequence is available on GenBank (accession number AF200268, Damgaard and Sperling 2001). A strict consensus tree shows the EF.D and EF.N lineages as monophyletic groups, but there is less than 50% bootstrap support for these lineages. Thus, the monophyly of the EF.D and EF.N lineages are not clearly established. Haplotypes W and FF do not group with either *dissortis* or *notabilis* lineages, and are found in a basal position to these 2 groups. Most of the *L. dissortis* alleles are unresolved in a strict consensus tree and are found in all populations except at site 9 in Maple Ridge, British Columbia. The tree had a homoplasy index of 0.677. The phylogeny suggests a distinct lineage of EF.D and EF.N haplotypes found within eastern and western populations, respectively, with geographically intermediate populations possessing haplotypes of both lineages.

ITS-1

All of the base pairs were sequenced for the 252 bp or 259 bp ITS 1 segment. Eight haplotypes were found among 99 *Limnoporus* specimens representing 10 populations. Table 2-9 summarizes the variable nucleotide positions in 8 ITS 1 haplotypes. One individual from population 8 was not included because multiple, overlapping bands on the sequence chromatograms were difficult to interpret. The uncorrected p-distance between haplotypes was between 0.004 and 0.020 (mean= 0.011). There were a total of 14 variable positions, including the 7 bp insertion. Each indel bp position was counted as one difference.

No interspecific heterozygotes were detected (Table 2-8), although sequences revealed intraspecific heterozygotes in five specimens. Phylogenetic analysis showed haplotype A to be *L. dissortis*-like (Figure 2-5) and it was found in all populations except population 9. It represented 80% of all haplotypes. Phylogenetic analysis showed haplotypes G and H to be *L. notabilis*-like. They were found mostly in the western populations 8 and 9, and represented 10% of haplotypes in population 1. Average nucleotide diversity within populations ranged from 0.0000 (sites 3, 5 and 7) to 0.0063 (site 8) (Table 2-6).

The proportion of shared haplotypes was equal to or greater than 0.79 for all pairwise population comparisons between sites 0 to 8 (Table 2-5). Site 9 did not share any haplotypes with other populations, except at sites 1 and 8. An exact test of differentiation of genotype frequencies was significant over all populations ($P=<<0.001$).

A test of differentiation among all pairs of sample sites showed significant differences ($p<.05$) in 35 out of 45 comparisons (Table 2-5). Most of the non-significant comparisons were found in comparisons between sites east of, and including site 8.

The main transition between *L. dissortis*-like haplotypes and *L. notabilis*-like haplotypes occurs between sites 7 and 9, over a distance of more than 900 km. In general, these results indicate that *L. dissortis*-like haplotypes are distributed relatively continuously from Niton Junction, Alberta (site 0) to site 8 just west of Jasper National Park, and *L. notabilis*-like haplotypes in this study were geographically restricted to site 8 and a population in Maple Ridge, British Columbia (site 9) (Figure 2-5).

A phylogeny of 8 ITS 1 alleles, including the individuals used to initially screen for variation, rooted with *L. rufoscutellatus*, resulted in 8 most-parsimonious trees (Figure 2-5). I generated the *L. rufoscutellatus* sequence using the same ITS 1 primer pairs previously described (GenBank accession number pending). A strict consensus tree had a homoplasy index of 0.136 and length 22. The topology separates 2 paraphyletic *L. notabilis* haplotypes, G and H, from a monophyletic *L. dissortis* group. The *L. dissortis* cluster was associated with all populations except site 9 and was supported by a bootstrap value of 80%. The *L. notabilis* cluster is associated with western populations of site 8 and 9, representing 100% of the Maple Ridge population, and 33% of the population west of Jasper National Park. Phylogenetic analysis reveals a geographically unrestricted distribution of haplotypes in the *L. dissortis* cluster with the exception of the western-most population. Haplotypes within the *L. notabilis* cluster clearly separate from this group and are geographically more restricted.

Gene flow estimates

A phylogeny of CO1 haplotypes without outgroups resulted in 863 most-parsimonious trees. I calculated the minimum number of migration events (s) for the first 20 trees, resulting in s-values of 33 or 34. Estimates of gene flow using the Slatkin and Maddison (1989) approach were 1.2 (95% C.I. of 0.7-1.8, s=33) and 1.3 (95% C.I. of 0.8-2.2, s=34) after 1000 replicates. A phylogeny of EF1-α haplotypes without an outgroup resulted in 100 most-parsimonious trees. The minimum number of migration events (s) was calculated for the first 20 trees, resulting in a range of s-values from 38-39. The estimate of gene flow for both s-values was 0.9, with 1000 repetitions and 95% confidence intervals of 0.6-1.3 (s=38) and 0.7-1.3 (s=39). The cladistic anlysis of gene flow was not applied to the ITS-1 phylogeny, because of the unresolved topology of the tree.

Gene flow estimates using Φ_{ST} values followed similar patterns for CO1 and EF1-α, but resulted in lower estimates. Gene flow estimates were greatest for CO1 (Nm=0.385), followed by EF1-α (Nm= 0.333) and ITS-1 (Nm=0.142).

To determine the relative extent of gene introgression, allele frequencies for each pair of markers were plotted against each other (Figure 2-6). A straight line was drawn through points representing "pure" populations (i.e. at ends of the transect) such that deviations above or below this line suggest, respectively, a greater or lesser extent of introgression. Pairwise plots show the allele frequency transitions between loci and generally agree with patterns of gene flow for EF1-alpha and CO1; introgression at these

2 loci occur at approximately equivalent rates. In contrast to the gene flow estimate generated by Φ_{ST} values, greater rates of ITS-1 introgression are evident in comparison with both CO1 and EF1-alpha.

Hybrid Index

Multi-locus associations show that populations east of Hinton (populations 0-4) contain individuals that are fixed for *L. dissortis*-type alleles at all 3 loci, with 2 exceptions (Figure 2-7). In total, six populations displayed at least one allele from both species. Three of these populations (1,3,6) were characterized entirely by *L. dissortis* alleles with the exception of an individual homozygous for an *L. notabilis* ITS 1 allele in population 1 and an EF1-α heterozygote in populations 3 and 6. Intermediate levels of hybridization were seen in populations 5, 7 and 8. With random mating and no differential selection, hybrid index scores from a mixed population should cluster in the middle of the histogram. The proportion of hybrid individuals (i.e. individuals with hybrid index scores between 2-4) was 0.5 (population 5), 1.0 (population 7) and 0.8 (population 8). Populations 7 and 8 contained hybrids with intermediate hybrid index scores (2-3) that tended to cluster towards the centre, while most of the hybrids in population 5 possessed genotypes characteristic of *L. dissortis* with the exception of 1 allele. In mixed populations, there were no signficant deviations from Hardy-Weinberg expectations at the EF1-α locus. Only population 8 contained ITS 1 alleles from both species and allele frequencies were significantly out of Hardy-Weinberg equilibrium ($p=0.004$), reflecting the lack of ITS-1 heterozygotes in this population (Table 2-8).

Measures of linkage disequilibrium in population 8 support the patterns provided by the hybrid-index scores and single locus data for population 8. There was no significant effect of linkage disequilibrium (Fisher's exact test, p=0.52) suggesting that these genes are assorting randomly.

Out of 12 males with an intermediate hybrid index score, 10 carried mtDNA from *L. notabilis*. Fourteen females had an intermediate hybrid index score, and 8 carried mtDNA from *L. notabilis*.

Cytonuclear disequilibrium

Populations 5, 7 and 8 were pooled for calculations of cytonuclear disequilibria because only these populations showed intermediate levels of hybridization (Figure 2-7). Significant cytonuclear disequilibria characterizes the pooled populations of 5, 7 and 8 at the EF1-α locus for D and D_3, but not at the ITS 1 locus (Table 2-10). At the EF1-α locus, the gametic phase disequilibrium, D, is positive and significant (Fisher's exact test, p=0.0008) and D_3, a measure of disquilibrium between *notabilis* genotypes (*NN*) and *dissortis* mtDNA(*d*) is negative and significant with Fisher's exact test (p=.002). Thus, individuals that are homozygous for *L. notabilis* alleles at the EF1-α locus are associated less commonly with *L. dissortis* mtDNA than that expected with a random model (Figure 2-8). D_1, a measure of disquilibrium between *dissortis* nuclear genotypes (*DD*) and *dissortis* mtDNA (*d*), is positive but non-significant (p=.08). There is no evidence for asymmetric direction in interspecific matings at the EF1-alpha locus although significantly higher sample sizes (n=172) are necessary to detect significance in D_2 with 90% power

($D_2 = .056$, $p=.23$). Conversely, associations of homozygous *L. dissortis* or *L. notabilis* ITS 1 alleles with *L. dissortis* mtDNA are consistent with expectations of random mating (Figure 2-9). Lack of heterozygotes at the ITS 1 locus precluded analysis for D_2.

DISCUSSION

Sequence data from one mitochondrial and two nuclear coding genes in *Limnoporus dissortis* and *L. notabilis* describe the population structure in 10 populations adjacent to and within a hybrid zone. The spatial distribution of 58 CO1 haplotypes, 27 EF1-α haplotypes and 8 ITS 1 haplotypes, combined with phylogenetic analysis using parsimony, provides evidence of ongoing asymmetrical hybridization and introgression. Strong barriers to gene exchange for mitochondrial DNA, relative to at least one of the nuclear genes, is consistent with reduced dispersal for females across the hybrid zone. Thus, incompatabilities due to cytoplasmic factors, like mtDNA, in a foreign genetic background may be a sigificant factor affecting hybrid female fitness. Fewer associations of *L. dissortis* mtDNA with *L. notabilis* nuclear alleles than expected under a random mating hypothesis, at least at the EF1-α locus, suggest that selection prevents *L. dissortis* mtDNA from introgressing into a *L. notabilis* nuclear background. A hypothesis of unidirectional hybridization is not strongly supported by an estimate of cytonuclear disequilibrium. In combination, these results provide new evidence to support a hypothesis of introgression mediated by functional hybrid males (Spence 1990, Sperling and Spence 1991) and suggest that most barriers to gene flow in mtDNA occur over a geographic distance of less than 170 km.

Structure and maintenance of the hybrid zone

Shared mtDNA, EF1-α and ITS-1 haplotypes among populations could result from introgession due to hybridization or from ancestral polymorphisms. If populations are sampled during a polyphyletic or paraphyletic stage of stochastic lineage sorting, it is possible to obtain shared genes between 2 species that are not necessarily due to hybridization (Pamilo and Nei 1998). Retained ancestral polymorphisms may be represented by two EF1-α haplotypes, FF and W, since they have substitutions characteristic of both species at two diagnostic positions and occupy a basal phylogenetic position. Their distribution at opposite ends of the sampling transect further supports an ancestral state hypothesis.

However, sharing of ancestral polymorphisms seems an unlikely explanation for overall patterns of haplotype distribution because phylogenetic analysis of haplotypes shows that there are clear divergences between *L. dissortis* and *L. notabilis* haplotypes at all 3 loci, although nuclear gene trees are not necessarily monophyletic. In addition, populations with haplotypes from both species lineages or clusters are located in the geographic transition area between species distribution ranges. Thus, patterns of haplotype distribution across this hybrid zone, together with evidence from allozymes (Sperling and Spence 1991) and morphology (Klingenberg et al. 2000) strongly point to hybridization and introgression as the cause of observed variation.

The number of CO1 haplotypes estimated in this study is higher than the number of CO1 haplotypes estimated for two species of *Potamobates* water striders in the Amazon (Galacatos et al. 2002). However, our haplotypes were derived from a longer

CO1 sequence so a greater amount of haplotype variation is not surprising. Andersen et al. (2000) sequenced 788 bp of CO1 in 5 species of marine *Halobates* sea skaters and found 49 haplotypes among 66 specimens. For some species, almost as many haplotypes were discovered as individuals sequenced. In general, the high number of *Limnoporus* CO1 haplotypes reported in this study is a reasonable estimate compared to other population studies of CO1 sequence variation in water striders.

That most CO1 haplotypes were found within just one population shows that mitochondrial genotypes have not spread very far. However, within each species lineage, at least one CO1 haplotype was relatively widespread. This pattern might reflect historically intermediate levels of gene flow within populations of *L. notabilis* and *L. dissortis*, with little introgression between species. It is possible that presumed ancestral genotypes for each species occur over a wide area but newer genotypes have not spread throughout the range of the species (Avise et al. 1987).

Permeability of species boundaries in this hybrid zone varies depending on the marker; mtDNA markers reveal a relatively narrow zone of 170 km or less, while nuclear markers appear to introgress over a broader zone. Many hybrid zones can be explained as a dynamic balance between dispersal into the zone and intrinsic selection against hybrids (Barton and Hewitt 1985). The width of these 'tension zones' is determined by the ratio of the dispersal rate and the effective strength of selection against hybrids at the centre of the cline. Thus, a broader cline is produced when dispersal is great relative to selection, with the cline narrowing as selection increases. Precise estimates of dispersal rates in *Limnoporus* are not known, although conservative estimates are in the order of

several kilometres (Spence 2000, Fairbairn and Butler 1990). Parental dispersal into the zone, and strong selection against hybrids (Sperling et al. 1997), can account for a width of 170 km for the mtDNA cline and the roughly concordant changes in EF1-α. Similar patterns of geographic variation and rates of gene flow for both EF1-α and CO1 markers are clearly not the result of genetic linkage, but could arise from similar selection pressures.

The wider ITS 1 cline cannot be explained as a tension zone, unless unrealistically great dispersal distances and/or weak selection pressure are invoked. It is more likely the latter situation. Widely distributed *dissortis*-like ITS 1 markers suggest an on-going westward sweep of neutral *L. dissortis* markers into *L. notabilis* populations. Given the relatively sharp mtDNA cline relative to nuclear markers (especially ITS 1) it seems most probable that male hybrids are responsible for broad introgression of *dissortis* genes. Presumably, the increased permeability to species boundaries is due to neutral selection at the ITS 1 locus. Neutral introgression has also been observed in a broad hybrid zone between 2 species of katydids in the southern United States (Shapiro 1998). In theory, in cases of neutral introgression the parameters determining hybrid zone width are dispersal and time elapsed since secondary contact, rather than dispersal and selection against hybrids (N.H. Barton, pers. comm in Shapiro 1998).

Given the wide distribution of *L. dissortis* ITS 1 alleles, the relatively low apparent level of gene flow at this locus is surprising. This may result from the method of using Φ_{ST} to calculate gene flow; Φ_{ST} characterizes the variation between haplotypes in a single population relative to all haplotypes. Thus, very little variation between all

haplotypes, as is the case with ITS 1, will result in low estimates of Φ_{ST} and gene flow. Undoubtedly, caution should be taken when interpreting results of indirect estimates of gene flow because there are several biologically unrealistic assumptions in the mathematical model, such as no selection, no mutation, and equality of all populations in terms of number of individuals and contributions to the migrant pool (Whitlock and McCauley 1999).

A potential problem with using nuclear ribosomal genes like ITS 1 is a high level of nucleotide variation within individuals due to the presence of multiple gene copies (Hillis and Dixon 1991). For example, in ticks variation within individuals at the ITS 2 locus is 4%, which is almost half of the variation between ticks from separate geographic locations (Rich et al. 1997). My results show an opposite trend, with relatively low levels of variation both within individuals and within species. Genes in a gene family, like ITS 1, often evolve in concert, resulting in duplicated gene copies that are very similar within a species but show differences between species (Ridley 1993). My results can be explained if concerted evolution homogenizes ribosomal arrays within lineages to create low variation within individuals, as has been found for ITS 2 sequences in mosquitoes (Fritz et al. 1994, Wesson et al. 1992). As in my study, Honda et al. (1998) found little variation in ITS 1 sequences within species and a high degree of variation between species of bugs in the genus *Orius*.

Another possibility is that low intraspecific variation and absence of ITS 1 heterozygotes can arise as a PCR artifact. Vazquez et al. (1994) tested the ability of PCR amplification to detect a 5 bp insertion/deletion distinguishing 2 grasshopper subspecies

in F_1 hybrids. Analysis by electrophoresis revealed that both alleles of a noncoding nuclear DNA fragment were not always amplified in known heterozygotes because of stochastic differences in the PCR amplification. The degree to which this may have influenced our results is not clear. Vasquez et al (1994) repeated PCR amplifications in triplicate before 100% of all heterozygotes were detected. Cloning of the fragments is necessary to resolve this uncertainty. Although some PCR amplifications were repeated in this study to verify unclear sequence chromatograms, they were not repeated in a systematic way. Cloning was not done, to mimimize time and expense.

Disequilibrium within the hybrid zone

The absence of pure parentals and the classification as hybrids of all individuals within at least one population suggests extensive interbreeding with little reproductive isolation upon contact. This is supported by tests of single locus Hardy-Weinberg equilibrium, with one exception. Although a population can reach Hardy-Weinberg equilibrium after only one generation of random mating, hybridization has been occuring for at least the past 16 years along the east slopes of the Rocky Mountains (Spence 1990). In 1984, Sperling and Spence (1991) found significant deviations from Hardy-Weinberg equilibrium at 3 autosomal allozyme loci in this region. It is important to note that hybridized populations near Jasper National Park appear to be more unimodal than bimodal in their genotypic distributions, while the Hinton population appears to be skewed towards pure *L. dissortis* and hybrids with mostly *L. dissortis* alleles.

Modality of genotypes can be used to infer strength of prezygotic barriers to gene flow and the type of selection acting on populations (Harrison and Bogdanowicz 1997).

For example, unimodal hybrid zones characterize 'hybrid swarms', where prezygotic barriers to gene flow break down and selection against hybrids is mostly endogenous (Jiggins and Mallet 2000 and references therein). Unimodal hybrid zones that are at or close to Hardy-Weinberg equilibrium have been reported between races of *Podisma* grasshoppers (Hewitt et al. 1987), species of *Papilio* butterflies (Sperling 1987), *Bombina* toads (Syzmura and Barton 1991), *Chorthippus* grasshoppers (Hewitt 1993) and colour pattern races of *Heliconius* butterflies (Mallet et al. 1998). In contrast, bimodal hybrid zones result from strong prezygotic isolation by assortative mating, for example, with an increased effect of exogenous or environmental selection (Jiggins and Mallet 2000 and references therein).

We did not detect linkage disequilibrium in the one population where the sample sizes and variation allowed a test. Unfortunately, very large sample sizes are required to reliably detect linkage disequilibrium (Brown 1975).

Genetic incompatabilities and selection against female hybrids

Selection against female hybrids is probably an important factor in this hybrid zone. Evidence from two previous studies indicate that female hybrids are at a selective disadvantage. In interspecific laboratory crosses, the surviving offspring are almost all male, suggesting that most female F1 hybrids fail to develop (Spence 1990). The second study, using allozyme data from field populations, revealed a significant deficiency of heterozygotes at an X-linked locus, G6PD (Sperling and Spence 1991). Females possess two X chromosomes in *Limnoporus* (Spence and Maddison 1986) and developmental failure may be associated with either the presence of an X chromosome of both species or

because of incompatabilities between cytoplasmic elements (e.g., mtDNA) and an X chromosome from the opposite species.

In our study, it is not likely that ITS 1 or EF1-α are X-linked genes. Sex-linked genes are thought to have the greatest effect on hybrid inviability and sterility (Coyne and Orr 1989), and therefore show little introgression in hybrid zones. Although X-linkage may account for the steep transition at the EF1-α locus, the finding of 7 interspecific heterozygote females is inconsistent with expectations of selection against female hybrids (Spence 1990, Sperling et al. 1997). Conversely, lack of heterozygotes at the ITS1 locus may suggest sex-linkage, but extensive introgression at this locus does not support this hypothesis. Alternatively, loci found in a heterozygous state may appear in hybrids that were created from multiple backcrossing events. Therefore, new genotypes created through backcrossing can appear heterozygous at some loci yet not suffer from incompatibility problems.

It is likely that individuals with an intermediate hybrid index score probably result from backcrossing to hybrid males. How selection acts on backcrossed individuals and surviving female hybrids in the field is unknown, although backcrossed hybrids have been produced in the laboratory for several generations (Spence, personal communication) with no obvious signs of decreased fitness.

The idea that mtDNA is a good marker of species boundaries is supported in studies of several taxon pairs of Lepidoptera (Sperling 1993, Sperling 1994, Prowell 1998, Sperling 2001), where selection against female hybrids can be explained on the basis of Haldane's rule (1922). Because females are the homogametic sex in *Limnoporus*, it is

necessary to invoke other mechanisms to explain a selective disadvantage to female hybrids. Our data raise the possibility that for females, selection favours some cytoplasmic and nuclear combinations over others. At the EF1-α locus, 6 out of the 7 hybrid females had *L. dissortis* mtDNA, although when all hybrid females with an intermediate hybrid index score are considered, only 6 out of 14 have *L. dissortis* mtDNA. Asymmetries in reciprocal crosses were demonstrated by Spence (1990), who could produce no females from interspecific crosses involving *L. notabilis* females, and only a few from crosses with *L. dissortis* females. Differential survival in female hybrids could be explained if the cytoplasm of some *L. dissortis* females was more tolerant of "foreign" genes. Nuclear-cytoplasmic epistatic effects occur in *Drosophila* (Hutter and Rand 1995, Kilpatrick and Rand 1995) and may explain lack of mtDNA introgression in our study.

Male mating tactics

Asymmetry in male interspecific mating success is not supported by EF1-α estimates of cytonuclear disequilibrium. However, backcrossed hybrids may be more likely to mate with an *L. notabilis* female. 10 out of 12 males and 8 out of 14 females with intermediate hybrid index scores possessed mtDNA from a *L. notabilis* female. In addition, there were fewer individuals with EF.N lineage haplotypes and mtD lineage haplotypes than predicted by random mating. Our results, although inconclusive, do not rule out a role for mating tactics as an important factor in unidirectional hybridization (Wirtz 1999).

Associations between body size and mating tactics may contribute to the asymmetrical flow of *L. dissortis* genes into *L. notabilis* populations. *L. dissortis* males are less discriminatory in their choice of mates, and in hybridized populations are more likely to be 'sneaky patrollers' who mate without any obvious premating display (Spence and Wilcox 1986). Conversely in mixed populations, *L. notabilis* males are more likely to send a courtship signal in the form of ripple signals (Spence and Wilcox 1986). Choice of mating tactic is associated with body size in mixed populations; smaller *L. dissortis* males are more likely to use sneak copulations than the larger bodied *L. notabilis* males. To the extent that females accept these tactics, more hybridization events should involve smaller-bodied males.

Habitat-dependent selection

The clinal transition pattern observed for all 3 markers could also result from selection for different genotypes along an environmental gradient. The concept of a mosaic distribution of multi-locus genotypes within hybrid zones (Harrison 1986) suggests that species-specific preferences for some features of the environment can result in patchily distributed genotypes across a heterogenous landscape. At the scale of the present study, we did not detect major reversals in cline shape as predicted if species responded differently to environmentally patchy features. *Limnoporus dissortis* and *L. notabilis* probably do not prefer substantially different habitat types, although the distribution and quality of habitats may influence patterns of hybridization (see Chapter

4). Selection for different habitat types can be studied with reciprocal rearing experiments of hybrid crosses in different natural environments.

Population 6 presents one instance of apparent patchiness, since it was mostly fixed for *L. dissortis* alleles but was flanked by populations showing more extensive hybridization. This is more likely the result of small sample sizes and the stochastic effects produced by seasonal colonization of ephemeral water bodies, rather than a species specific response to habitat features of population 6. *Limnoporus* water striders are found in shallow, temporary water bodies such as roadside ditches and flight between water bodies is a regular part of their life history (Spence 2000). The first colonists to reach these habitats could determine the genetic composition of populations, possibly creating a mosaic-like distribution of genotypes (see Chapter 3). Dispersal between patchily distributed habitats may account for a mosaic distribution of hybridizing fire ants (Shoemaker et al. 1996) and unusually broad clines in a katydid hybrid zone (Shapiro 1998).

Studies of genetic incompatabilities alone are not sufficient to understand the processes occurring within hybrid zones. Further studies can explore mechanisms associated with the apparent movement of this hybrid zone (Klingenberg et al. 2000) and landscape features that may contribute to patterns of reproductive isolation. With the development of diagnostic nuclear DNA markers used in this study and mtDNA markers we can now more clearly address these questions.

Table 2-1: Geographic distribution of individuals screened for diagnostic nucleotide variation and summary results.

Species	Collection Location	CO1	EF1-alpha	ITS 1
L. dissortis	Grande Prairie, northern AB	x	x	x
L. dissortis	George Lake, central AB	x	x	x
L. dissortis	Bonnyville, eastern AB	x	x	x
L. dissortis	Gatineau Hills, PQ	x	x	x
L. notabilis	Petaluma, California	x	x	x
L. notabilis	Vancouver, BC	x	x	x
L. notabilis	Revelstoke, BC	x	x	x
L. notabilis	Fernie, BC		x	x
	# bp sequenced:	819	487	252/259
	# diagnostic nucleotide sites:	11	2	14

Table 2-2: Location of populations sampled to assess variation across the *Limnoporus* hybrid zone.

Population #	n	Location	Latitude/ Longitude
0	10	Niton Junction, Alberta	53.617°N, 115.775 °W
1	10	Edson, Alberta	53.570°N, 116.494 °W
2	10	22 km west of Edson, Alberta	53.558°N, 116.758°W
3	10	14 km west of population 2	53.544°N, 116.942°W
4	10	8.6 km west of population 3	53.539°N, 117.064°W
5	10	Hinton, Alberta	53.399°N, 117.586°W
6	10	25 km west of Hinton	53.235°N, 117.822°W
7	10	east side of Jasper National Park	53.115°N, 117.971°W
8	10	west of Jasper National Park	52.917°N, 118.791°W
9	10	Maple Ridge, British Columbia	49.305°N, 122.555°W

Table 2-3a: Percent sequence divergence at the CO1 locus between individuals collected from geographically distant populations. Interspecific divergences are shown in bold. 819 bp were sequenced.

	A George Lake Alberta	B Bonnyville Alberta	C Grande Prairie Alberta	D Gatineau Hills Quebec	E Revelstoke B.C.	F Vancouver B.C.	G Petaluma California
A	-						
B	0.49	-					
C	0.98	0.73	-				
D	0.85	0.61	0.61	-			
E	**2.08**	**1.83**	**1.71**	**1.59**	-		
F	**2.08**	**2.08**	**1.95**	**1.83**	0.24	-	
G	**1.83**	**1.59**	**1.71**	**1.71**	0.24	0.49	-

Table 2-3b: Percent sequence divergence at the EF1-alpha locus between individuals collected from geographically distant populations. Interspecific divergences are shown in bold. 487 bp were sequenced.

	A George Lake Alberta (i)	B George Lake Alberta (ii)	C Bonnyville Alberta	D Grande Prairie Alberta	E Gatineau Hills Quebec	F Revelstoke B.C.	G Fernie B.C.	H Vancouver B.C.	I Petaluma California
A	-								
B	0.41	-							
C	0.41	0	-						
D	0.41	0	0	-					
E	0.41	0	0	0	-				
F	**0.82**	**0.41**	**0.41**	**0.41**	**0.41**	-			
G	**0.82**	**0.41**	**0.41**	**0.41**	**0.41**	0	-		
H	**0.82**	**0.41**	**0.41**	**0.41**	**0.41**	0	0	-	
I	**0.82**	**0.41**	**0.41**	**0.41**	**0.41**	0	0	0	-

Table 2-3c: Percent sequence divergence at the ITS 1 locus between individuals collected from geographically distant populations. Interspecific divergences are shown in bold. Sequences were 252 or 259 bp long.

	A George Lake Alberta (i)	B George Lake Alberta (ii)	C Bonnyville Alberta	D Grande Prairie Alberta	E Gatineau Hills Quebec	F Revelstoke B.C.	G Fernie B.C.	H Vancouver B.C.	I Petaluma California
A	-								
B	0	-							
C	0	0	-						
D	0	0	0	-					
E	0	0	0	0	-				
F	**1.68**	**1.6**	**1.62**	**1.6**	**1.6**	-			
G	**1.25**	**1.18**	**1.2**	**1.18**	**1.18**	0	-		
H	**1.68**	**1.6**	**1.63**	**1.6**	**1.6**	0	0	-	
I	**1.68**	**1.6**	**1.62**	**1.6**	**1.6**	0	0	0	-

Table 2-4: Unique CO1 haplotypes and variable nucleotide positions within *L. notabilis* and *L. dissortis*. Numbers at top of columns indicate nucleotide positions relative to *Drosophila yakuba* sequence (Clary and Wolstenholme 1985). Haplotypes are arranged approximately by sequence similarity. Sequence variation is compared to Haplotype A, shown at the top of each page. Dots indicate the same base pair as shown for Haplotype A. Letters show where base pairs differ from Haplotype A.

	2199	2226	2227	2244	2250	2260	2272	2275	2278	2299	2300	2308	2311	2312	2314	2315	2326	2335	2344	2347	2350	2359	2431	2437	2449	2452	2467	2470
HaploA	T	T	G	A	A	G	T	A	A	A	T	T	A	T	A	T	T	A	A	T	A	G	C	A	T	T	C	T
HaploB	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploJ	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploL	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	.
HaploN	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C
HaploO	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploQ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.
HaploS	C	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.
HaploJJ	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.
HaploU	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.
HaploKK	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploV	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploX	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploY	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploZ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploAA	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.
HaploBB	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploCC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	.
HaploDD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HaploEE	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	C	.	.	.
HaploGG	C	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploHH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.
HaploII	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.

Table 2-4: continued (2 of 4)

	2199	2226	2227	2244	2250	2260	2272	2275	2278	2299	2300	2308	2311	2312	2314	2315	2326	2335	2344	2347	2350	2359	2431	2437	2449	2452	2467	2470
HaploA	T	T	G	A	A	G	T	A	A	A	T	T	A	T	A	T	T	A	A	T	A	G	C	A	T	T	C	T
HaploC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.
HaploE	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploG	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploI	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploK	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.
HaploM	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploLL	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	A	.	.	.	.	.	C
HaploP	.	.	A	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploR	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	C	.	.	.	.	A	.	.	.	.	.	C
HaploNN	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploMM	.	.	.	.	.	.	.	.	G	.	C	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	.
HaploFF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploOO	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
HaploPP	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploQQ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploRR	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploSS	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploTT	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploUU	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	.	C	C	.	.
HaploVV	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.
HaploWW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HaploBBB	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploCCC	.	.	.	.	.	.	.	.	G	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploDDD	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploEEE	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	A	.	.	.	.	.	C
HaploFFF	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploXX	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	G	.	.	.	.	.	T	C
HaploYY	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	A	C
HaploZZ	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C
HaploAAA	.	.	.	.	.	.	.	.	G	.	.	.	.	C	G	.	C	.	.	.	.	.	.	.	.	.	.	C

Table 2-4: continued (3 of 4)

	2479	2483	2491	2492	2507	2512	2518	2536	2539	2545	2596	2602	2605	2608	2611	2617	2620	2635	2641	2653	2662	2665	2674	2675	2689	2698	2722
HaploA	T	T	A	C	C	T	C	T	T	A	C	C	T	A	A	A	G	T	T	A	A	A	A	A	T	A	C
HaploB	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.
HaploD	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploH	.	C	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploJ	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploL	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploN	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploO	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploQ	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.
HaploS	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HaploT	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploJJ	.	.	.	.	T	C	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploU	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploKK	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploV	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploW	.	.	G	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploX	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploY	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.
HaploZ	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploAA	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploBB	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploCC	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploDD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploEE	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.
HaploGG	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HaploHH	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HaploII	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.

Table 2-4: continued (4 of 4)

	2479	2483	2491	2492	2507	2512	2518	2536	2539	2545	2596	2602	2605	2608	2611	2617	2620	2635	2641	2653	2662	2665	2674	2675	2689	2698	2722
HaploA	T	T	A	C	C	T	C	T	T	A	C	C	T	A	A	A	G	T	T	A	A	A	A	A	T	A	C
HaploC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HaploE	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HaploG	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploI	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploK	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploM	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploLL	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	.
HaploP	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploR	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	.
HaploNN	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	.
HaploMM	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	A	.	.	G	.	.	.	.	.	.	T
HaploFF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	.	.	.	.	.	.	.
Haplo00	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploPP	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploQQ	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploRR	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploSS	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T
HaploTT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploUU	.	.	.	.	T	.	A	C	.	G	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploVV	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
HaploWW	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.
HaploBBB	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	G	.	.	.	.	.
HaploCCC	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploDDD	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploEEE	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploFFF	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	T	.	.	.	.
HaploXX	.	.	.	.	T	.	T	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	.
HaploYY	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	.	G	.	.	.	.	.	.	T
HaploZZ	.	.	.	.	T	.	.	.	.	.	.	T	.	.	G	.	A	.	C	G	.	.	.	.	.	.	T
HaploAAA	.	.	.	.	T	.	.	.	.	.	.	T	.	G	G	.	A	.	.	G	.	.	.	.	.	.	T

Table 2-5: Population differentiation by haplotype frequency. Proportion of shared haplotypes is shown to the left of the diagonal. To the right of the diagonal are P-values for the pair-wise exact test of the random distribution of haplotype frequencies. The test was not possible for CO1 because haplotypes were highly polymorphic. '+' indicates population pairs where the test was not possible because only one haplotype was present in both populations. Population numbers are indicated along the top and side of the grid. Darkened grid lines block off populations that are characterized mostly by *L. dissortis* individuals (0 to 6), from populations 7 to 9.

CO1

	0	1	2	3	4	5	6	7	8	9
0	-									
1	0.00	-								
2	0.10	0.45	-							
3	0.10	0.25	0.25	-						
4	0.10	0.25	0.25	0.20	-					
5	0.00	0.00	0.00	0.00	0.00	-				
6	0.15	0.20	0.15	0.15	0.15	0.00	-			
7	0.00	0.00	0.20	0.00	0.00	0.00	0.00	-		
8	0.00	0.00	0.00	0.00	0.00	0.35	0.00	0.45	-	
9	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.30	0.30	-

EF1-alpha

	0	1	2	3	4	5	6	7	8	9
0	-	0.210	0.018	0.419	0.148	0.005	0.127	<.001	<.001	<.001
1	0.79	-	0.171	0.167	0.281	0.045	0.128	<.001	<.001	<.001
2	0.68	0.68	-	0.428	0.333	0.161	0.007	<.001	<.001	<.001
3	0.79	0.68	0.80	-	0.424	0.398	0.008	0.002	<.001	<.001
4	0.82	0.75	0.75	0.83	-	0.053	0.020	<.001	<.001	<.001
5	0.66	0.60	0.75	0.80	0.68	-	0.005	0.085	0.007	<.001
6	0.95	0.75	0.50	0.65	0.70	0.60	-	0.001	<.001	<.001
7	0.53	0.80	0.43	0.65	0.43	0.73	0.55	-	0.029	<.001
8	0.55	0.43	0.33	0.58	0.38	0.73	0.73	0.40	-	0.252
9	0.00	0.00	0.00	0.23	0.00	0.55	0.23	0.70	0.75	-

ITS 1

	0	1	2	3	4	5	6	7	8	9
0	-	0.042	0.108	<.001	0.914	<.001	0.101	<.001	<.001	<.001
1	0.80	-	0.042	<.001	0.040	<.001	0.037	<.001	0.178	<.001
2	0.90	0.80	-	<.001	0.104	<.001	0.110	<.001	<.001	<.001
3	0.95	0.85	0.95	-	<.001	+	<.001	+	<.001	<.001
4	1.00	0.80	0.90	0.95	-	<.001	0.110	<.001	<.001	<.001
5	0.95	0.85	0.95	1.00	0.95	-	<.001	+	<.001	<.001
6	0.90	0.80	0.90	0.95	0.90	0.95	-	<.001	<.001	<.001
7	0.95	0.85	0.95	1.00	0.95	1.00	0.95	-	<.001	<.001
8	0.79	0.89	0.79	0.80	0.79	0.84	0.79	0.84	-	<.001
9	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.65	-

Table 2-6: Average nucleotide diversity for each population, by gene. Sample size (n) indicates the number of gene copies.

CO1

site:	9	8	7	6	5	4	3	2	1	0
n	10	10	10	10	10	10	10	10	10	10
nucleotide diversity	0.0043	0.0040	0.0118	0.0036	0.0111	0.0083	0.0076	0.0053	0.0047	0.0010
(+/- S.D.)	0.0029	0.0027	0.0069	0.0025	0.0065	0.0050	0.0047	0.0034	0.0031	0.0059

EF1-alpha

site:	9	8	7	6	5	4	3	2	1	0
n	20	20	20	20	20	20	20	20	20	18
nucleotide diversity	0.0015	0.0030	0.0034	0.0022	0.0026	0.0019	0.0015	0.0027	0.0023	0.0006
(+/- S.D.)	0.0013	0.0020	0.0023	0.0017	0.0019	0.0015	0.0013	0.0019	0.0017	0.0007

ITS 1

site:	9	8	7	6	5	4	3	2	1	0
n	20	18	20	20	20	20	20	20	20	20
nucleotide diversity	0.0020	0.0063	0.0000	0.0007	0.0000	0.0007	0.0000	0.0007	0.0040	0.0007
(+/- S.D.)	0.0019	0.0043	0.0000	0.0011	0.0000	0.0011	0.0000	0.0011	0.0031	0.0011

Table 2-7: Unique EF1-α haplotypes and variable nucleotide positions within *L. notabilis* and *L. dissortis*. Numbers at top of columns indicate nucleotide positions relative to *Drosophila melanogaster* sequence (GenBank accession number X06859). Sequence variation is shown relative to Haplotype A; dots indicate the same base pair as for Haplotype A. Letters show where base pairs differ from Haplotype A. Haplotypes are arranged approximately by sequence similarity.

	2446	2470	2485	2515	2530	2536	2542	2543	2554	2653	2758	2788	2818	2869	2893	2908	2929
HaploA	A	C	C	T	C	C	C	C	G	C	C	C	C	C	T	C	T
HaploC	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HaploD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
HaploE	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
HaploF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
HaploG	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.
HaploH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.
HaploI	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
HaploJ	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HaploK	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
HaploL	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.
HaploM	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
HaploN	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.
HaploO	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.
HaploP	.	.	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.
HaploQ	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
HaploR	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
HaploS	.	T	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.
HaploT	.	T	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.
HaploU	.	T	.	.	.	.	.	G	.	.	.	T	.	.	.	.	.
HaploW	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.
HaploX	.	.	T	.	.	T	.	.	.	.	T	.	.	.	.	.	.
HaploY	.	.	.	C	.	.	.	.	.	.	.	T	T	.	.	.	.
HaploEE	.	T	.	.	T	.	.	G	.	.	.	T	.	.	.	.	.
HaploCC	.	T	.	.	.	.	.	G	.	.	.	T	.	T	.	.	.
HaploBB	.	T	.	.	.	.	.	G	.	.	.	.	.	.	A	.	.
HaploFF	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.

Table 2-8: Heterozygote deficit by locus for each population with alleles coded as either *dissortis*-like or *notabilis*-like. 'O'= observed number of heterozygotes, 'E'= expected number of heterozygotes. Only population 8 significantly deviated from Hardy-Weinberg equilibrium at the ITS 1 locus and significance ($p<.05$) is indicated with a '*'. Many populations were monomorphic for either allele type and could not be tested. These populations are indicated with a '-'.

Locus		0	1	2	3	4	5	6	7	8	9
EF1-α	O	-	-	-	0.100	-	0.300	0.100	0.500	0.111	-
	E	-	-	-	0.195	-	0.395	0.195	0.542	0.398	-
ITS-1	O	-	0.000	-	-	-	-	-	-	0.000*	-
	E	-	0.284	-	-	-	-	-	-	0.576	-

Table 2-9: Unique ITS 1 haplotypes and variable nucleotide positions within *L. notabilis* and *L. dissortis*. Numbers at top of columns indicate nucleotide positions within the 252 or 259 bp fragment. "-" indicates a gap. Dots indicate the same base pair as shown for a similar position in Haplotype A. Sequence variation shown relative to Haplotype A.

	6	61	75	99	108	109	110	112	113	115	141	174	231	243
HaploA	G	T	T	-	-	-	-	-	-	-	C	G	A	C
HaploB	T	.	.	-	-	-	-	-	-	-	.	.	.	.
HaploC	.	.	.	-	-	-	-	-	-	-	.	T	.	.
HaploD	.	.	.	-	-	-	-	-	-	-	.	A	.	.
HaploE	.	.	A	-	-	-	-	-	-	-	.	.	.	.
HaploG	.	G	A	T	C	A	A	A	A	G	.	.	T	T
HaploH	.	G	.	T	C	A	A	A	A	G	.	.	T	T
HaploF	.	.	.	-	-	-	-	-	-	-	T	.	.	.

Table 2-10: Estimates of cytonuclear disequilibria for 2 nuclear loci. Only EF1-α had signicant estimators with p-values < .05, indicated with at '*'.

Nuclear Locus	n	D	D_1	D_2	D_3
		Estimates of nuclear-cytoplasmic disequilibria			
EF1-α	30	0.106 +/- 0.000 *	0.078 +/- 0.038	0.056 +/- 0.041	-0.133 +/- 0.042 *
ITS-1	29	0.034 +/- 0.027	0.034 +/- 0.027	-	-0.034 +/ 0.027

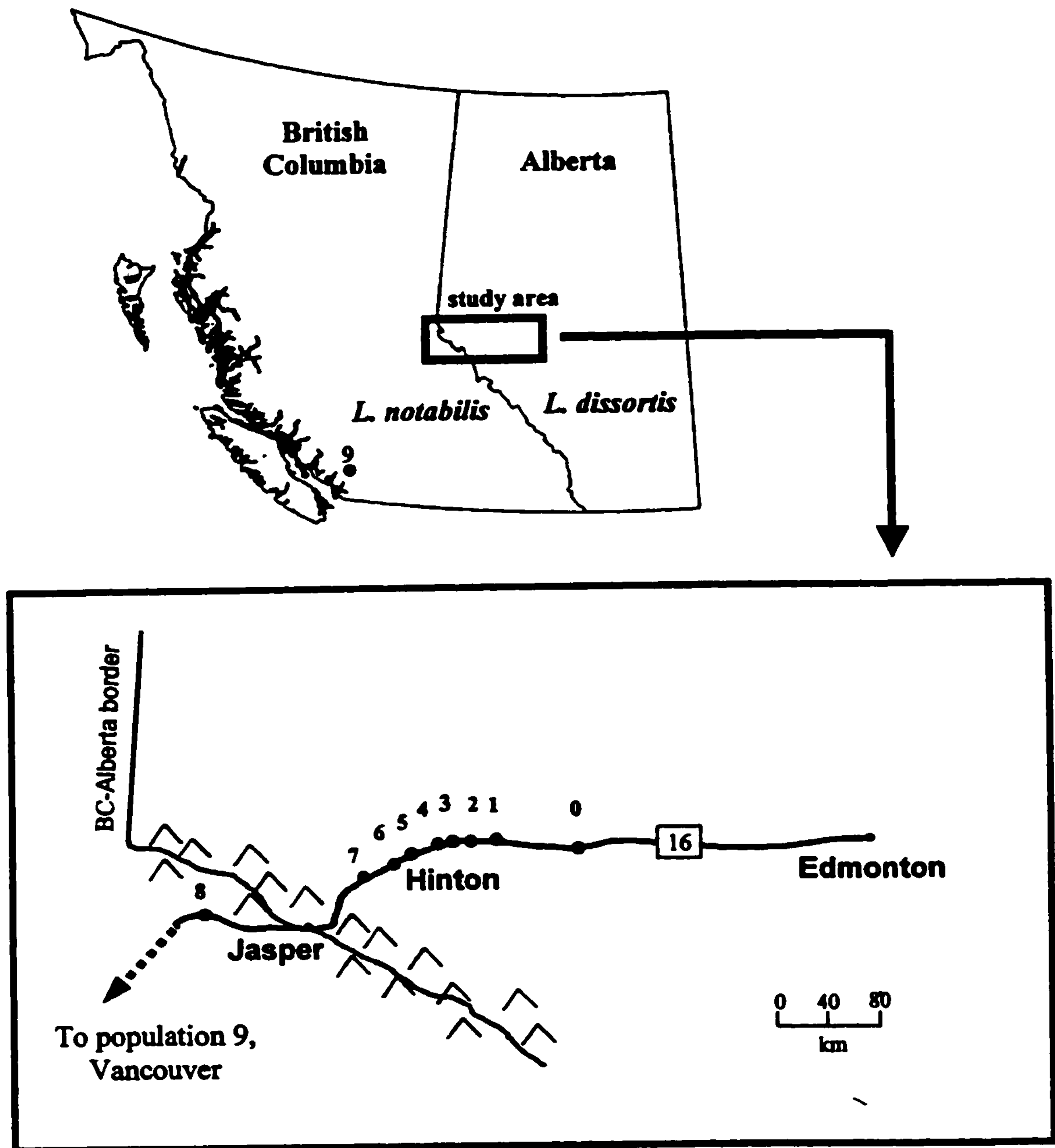

Figure 2-1: Schematic diagram of transect through hybrid zone shows populations sampled as circles with population number indicated above. Ten populations were sampled in total, with ten specimens per population. Highway number is shown in box.

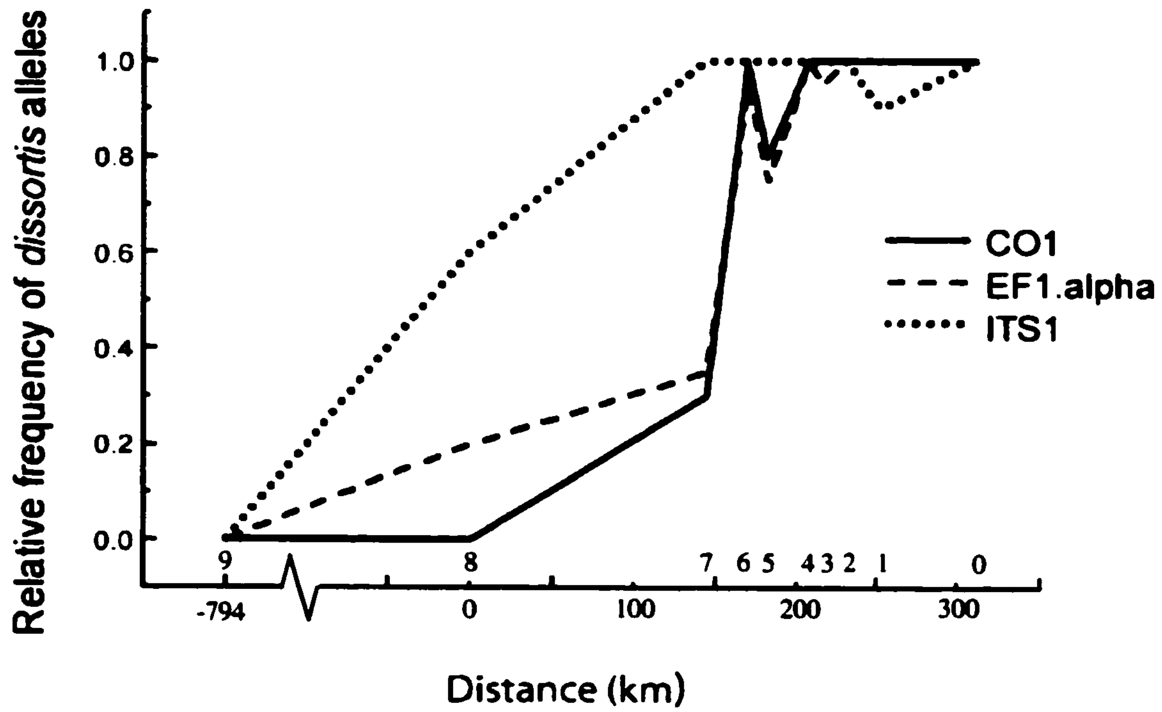

Figure 2-2: Relative frequency of *L. dissortis*-like alleles for 2 nuclear loci and one mitochondrial locus as a function of geographic distance. Locality number is indicated on the upper side of the x-axis.

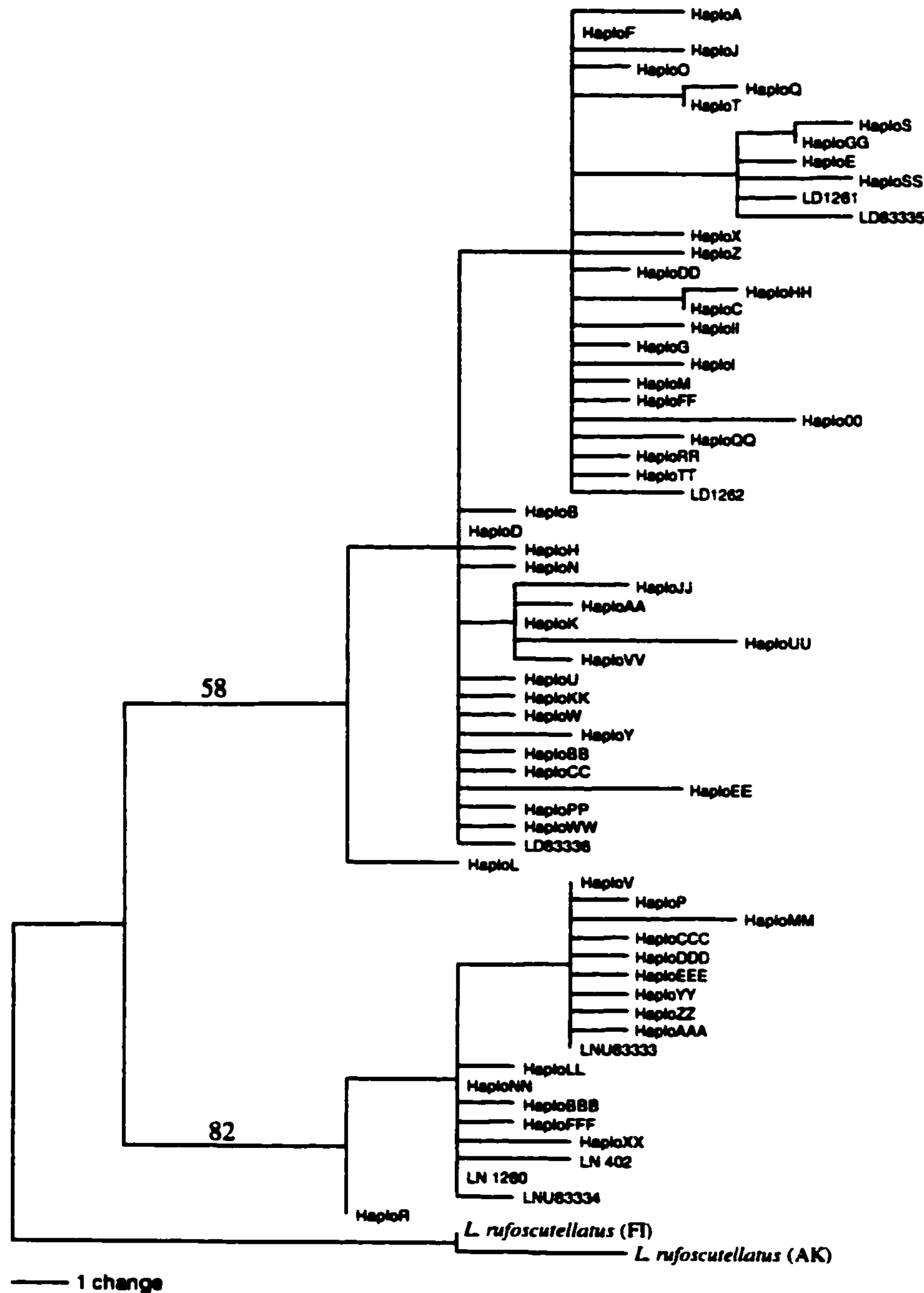

Figure 2-3a: Strict consensus phylogram from parsimony analysis of 58 mtDNA CO1 haplotypes and specimens used for initial screening of variation, rooted with *L.rufoscutellatus* (FI= Finland, AK=Alaska). *L. rufoscutellatus* sequences were available on GenBank (accession numbers U83337, U83338)(Sperling et al. 1997). Only bootstrap *values* >50% for the groups defining *L. dissortis* and *L. notabilis* lineages are shown. Figure 3b provides information on haplotype locations and frequencies.

Figure 2-3b: (next page) CO1 haplotype frequency and distribution. Haplotypes are ordered according to phylogram in Figure 3a, with major lineages drawn to the left of the grid. Population numbers are indicated at the top of the grid, ordered from west to east. For screened individuals (S), letters indicate collecting locality (GL=George Lake, B=Bonnyville, GP=Grande Prairie, GH=Gatineau Hills, M=Maple Ridge, CA=California, R=Revelstoke)

Figure 2-3b: See previous page (Figure 2-3a) for interpretation.

Haplotype	S	9	8	7	6	5	4	3	2	1	0
HaploA									1		
HaploF							1	1	2	4	
HaploJ									1		
HaploO								1			
HaploQ								1			
HaploT								1			
HaploS								1			
HaploGG							3				
HaploE										1	
HaploSS											1
LD1261	B										
LD83335	GL										
HaploX						2					
HaploZ						1					
HaploDD					1						
HaploHH							1				
HaploC							1				
HaploII							1				
HaploG										1	
HaploI										1	
HaploM					1					1	
HaploFF							1				
HaploOO											1
HaploQQ											1
HaploRR											1
HaploTT											1
LD1262	GP										
HaploB				2					2		
HaploD					2		1	1	1		1
HaploH									1		
HaploN								2			
HaploJJ								1			
HaploAA					1	1					
HaploK									2	1	
HaploUU											1
HaploVV											1
HaploU						1					
HaploKK						1					
HaploW						1					
HaploY						1					
HaploBB					4						
HaploCC					1					1	
HaploEE							1				
HaploPP											1
HaploWW											1
LD83336	GH										
HaploL								1			
HaploV		1	5	1		2					
HaploP				2							
HaploMM				1							
HaploCCC		1									
HaploDDD		2									
HaploEEE		1									
HaploYY			1								
HaploZZ			1								
HaploAAA			1								
LNU83333	CA										
HaploLL				1							
HaploNN		3		1							
HaploBBB		1									
HaploFFF		1									
HaploXX			1								
LN402	F										
LN1260	R										
LNU83334	M										
HaploR		1	2								

L. dissortis

L. notabilis

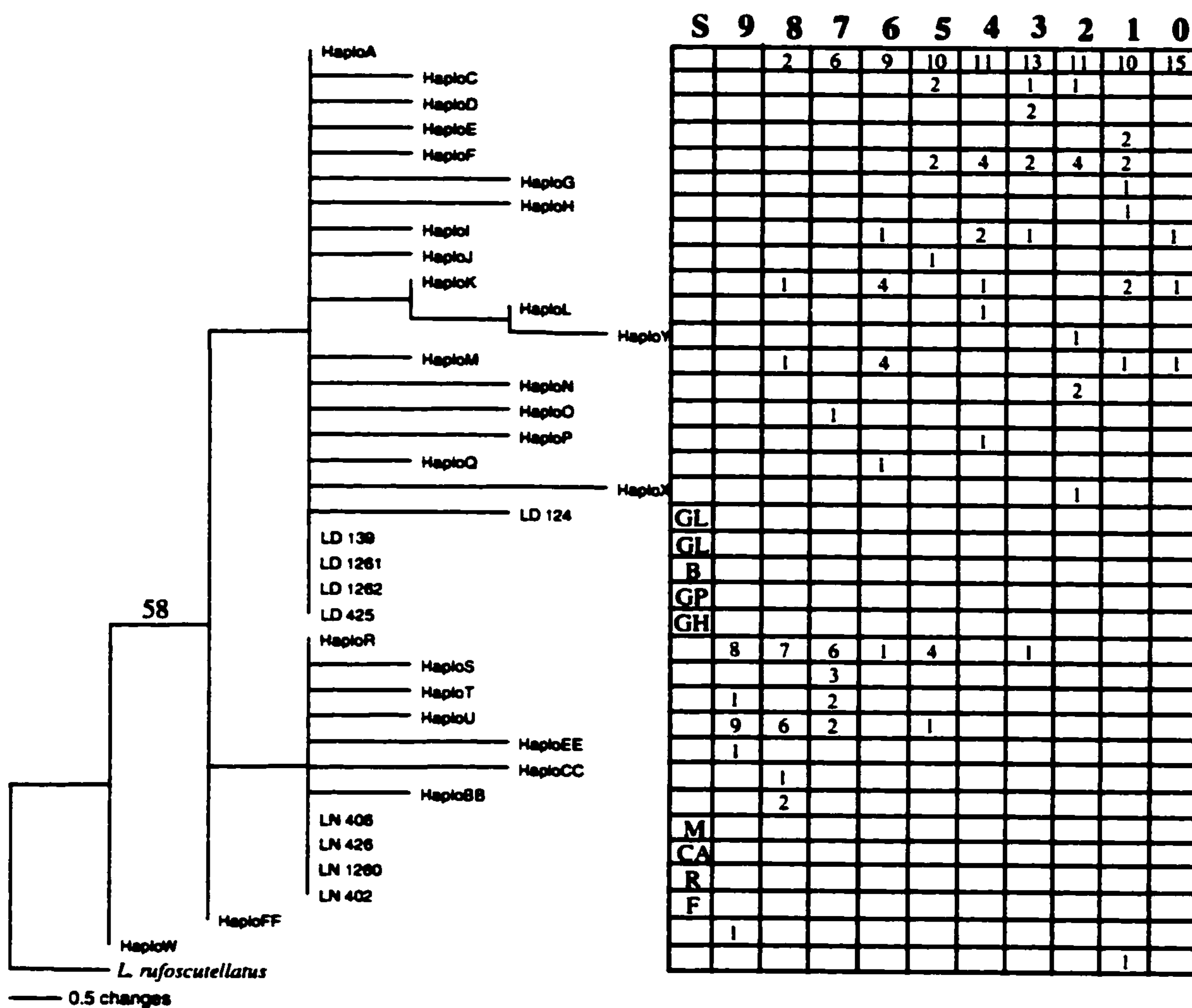

Figure 2-4: Strict consensus phylogram from analysis of 27 EF1-alpha haplotypes and specimens used for initial screening of variation, rooted with *L. rufoscutellatus* (GenBank accession number AF200268, Damgaard and Sperling 2001). Population numbers are indicated at the top of the grid, ordered from west to east. For screened individuals (S), letters indicate collecting locality (GL=George Lake, B=Bonnyville, GP=Grande Prairie, GH=Gatineau Hills, M=Maple Ridge, CA=California, R=Revelstoke, F=Fernie). Only bootstrap values >50% are shown

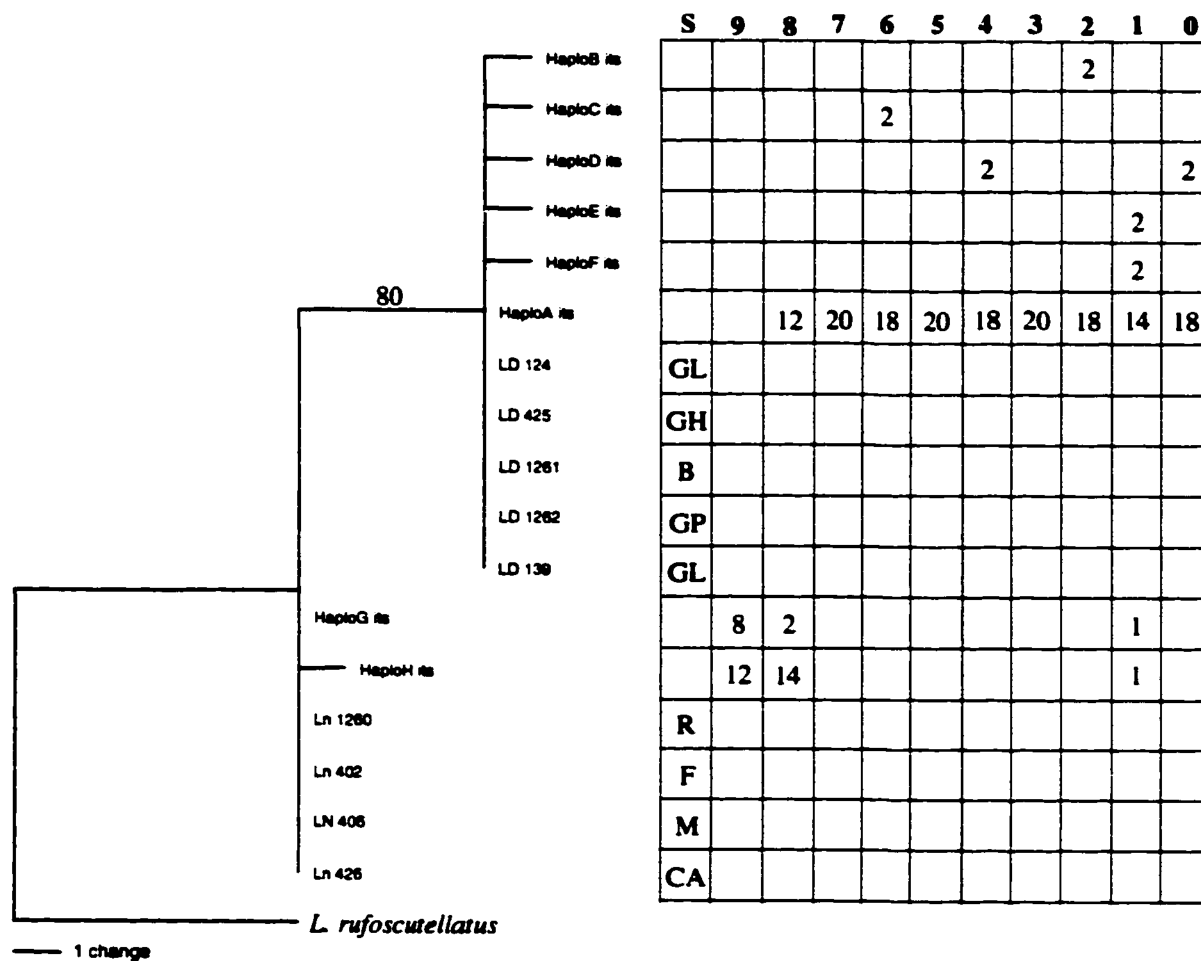

Figure 2-5: Strict consensus phylogram from parsimony analysis of 8 ITS 1 haplotypes and individuals used to initially screen for variation, rooted with *L. rufoscutellatus*. *L. rufoscutellatus* was sequenced using the same primer pair used for *L. notabilis* and *L. dissortis* (GenBank accession number pending). See Figure 4 for location codes. Only bootstrap values >50% are shown.

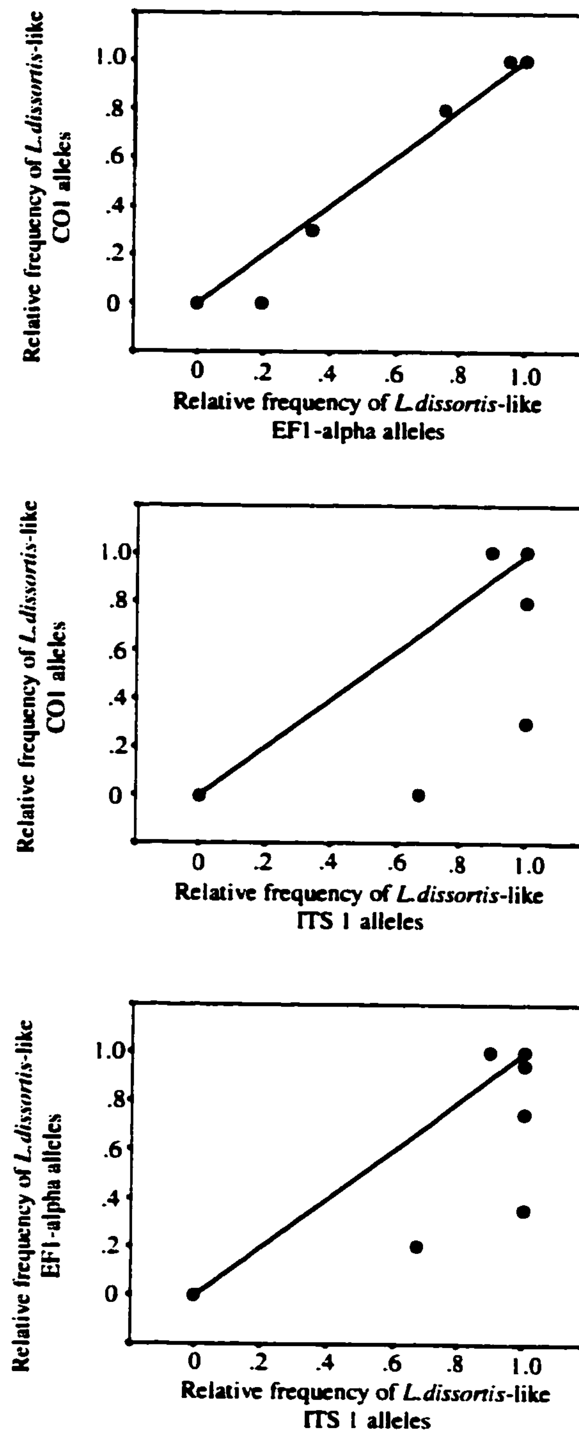

Figure 2-6: Pairwise comparisons of allele frequencies. The straight line connects 'pure' populations at either end of the transect such that values falling above or below this line indicate different rates of gene flow between loci. CO1 and EF1-alpha show similar rates of gene flow, while ITS 1 introgresses more readily than both loci.

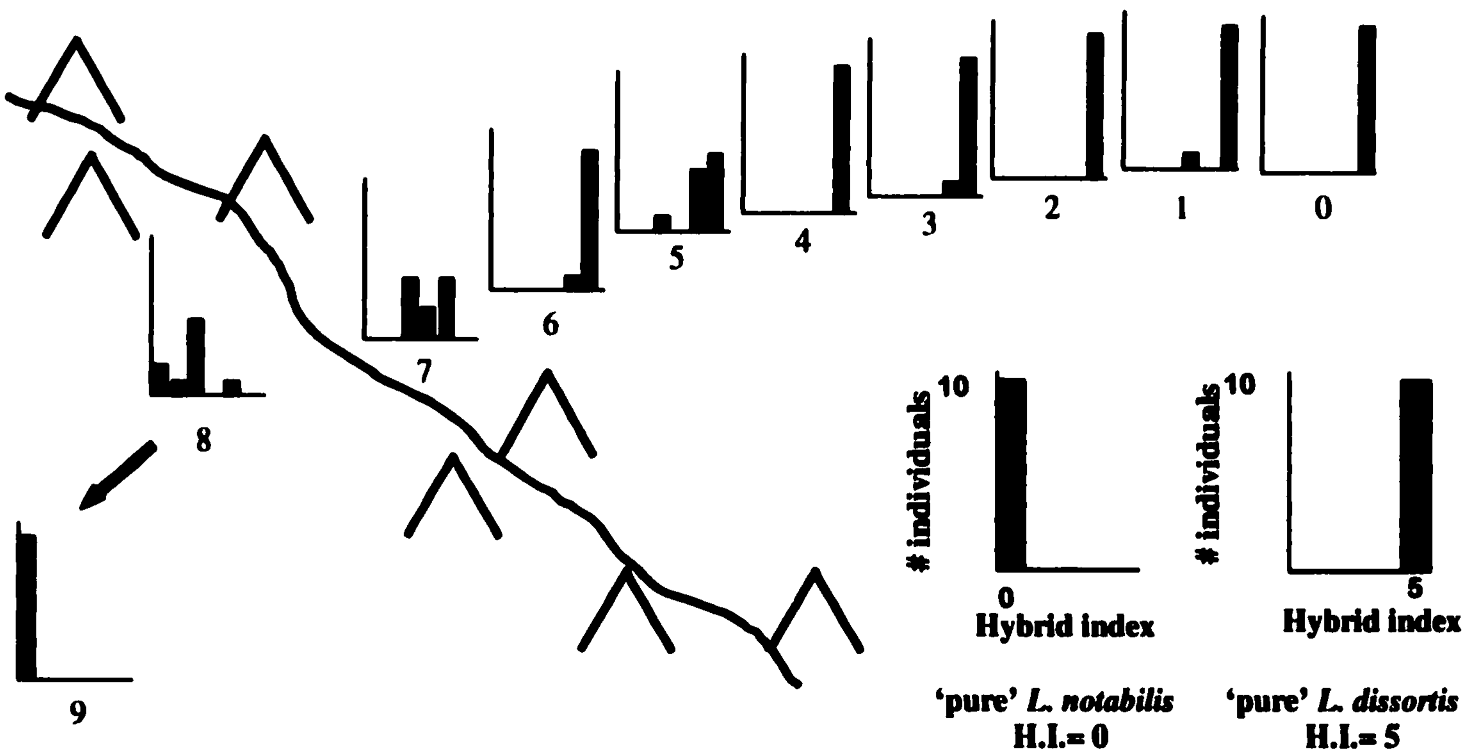

Figure 2-7: Distribution of hybrid index scores (H.I.) across the transect. One point was contributed to the hybrid index for every *dissortis* allele an individual possessed. Population number is shown below histograms. Enlarged histograms at right provide interpretation.

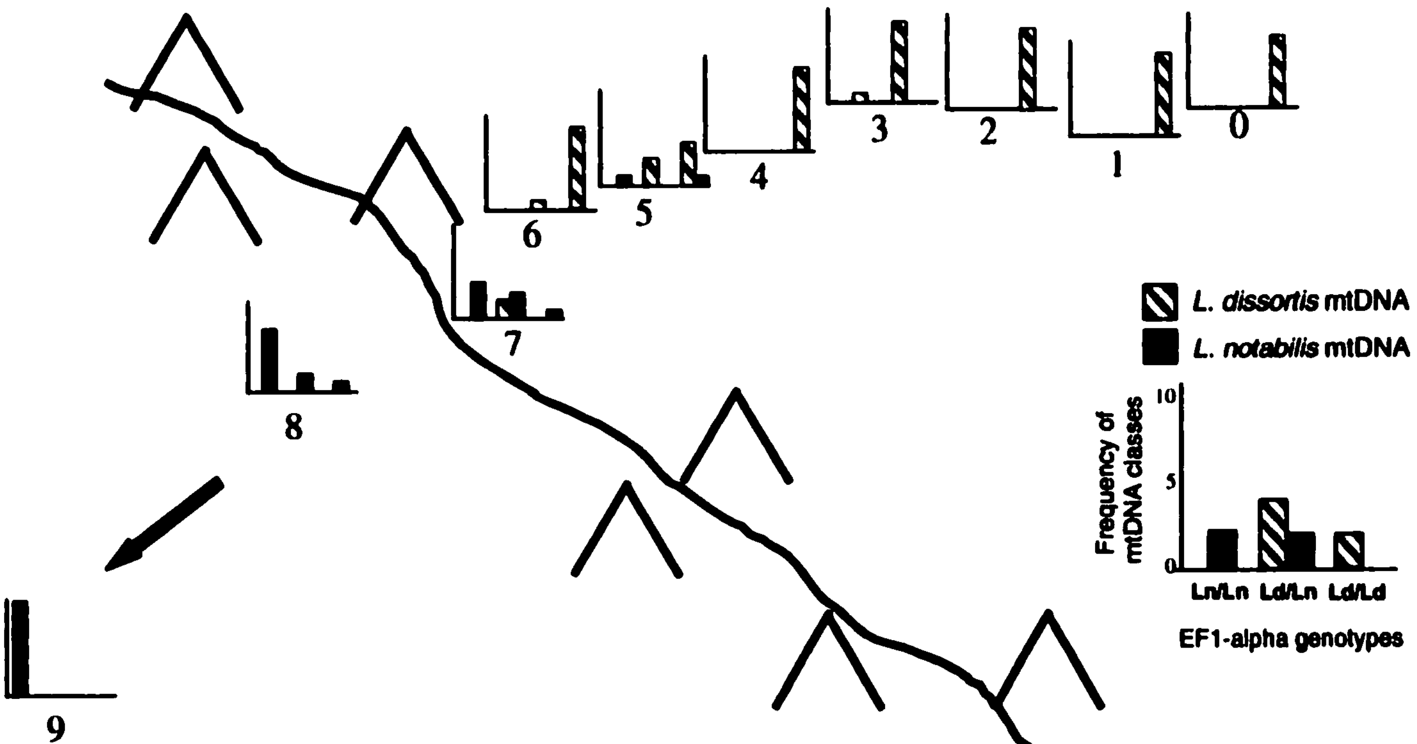

Figure 2-8: Frequency distributions of mitochondrial haplotypes as a function of EF1-alpha genotypes. Enlarged histogram at right provides interpretation. Population numbers are shown below histograms

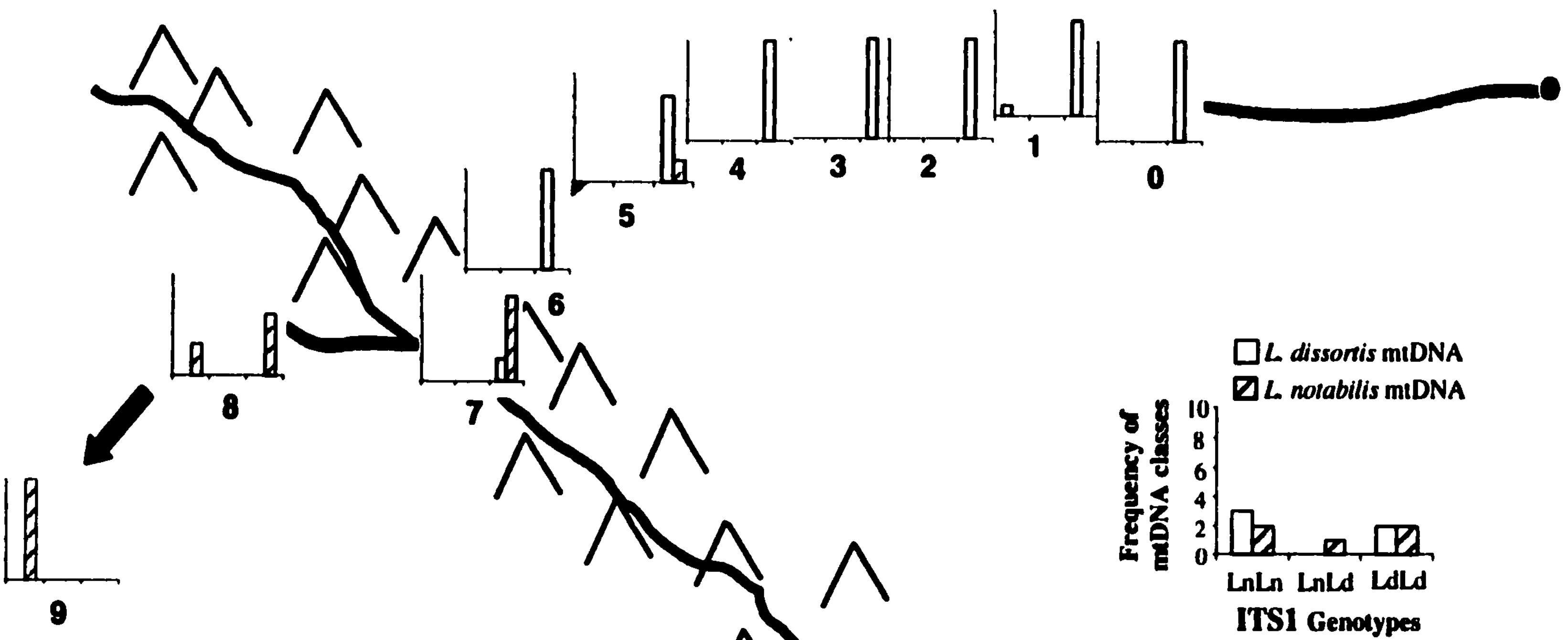

Figure 9: Frequency distributions of mitochondrial haplotypes as a function of ITS 1 genotypes. Enlarged histogram at right provides interpretation Site numbers are indicated below histograms.

REFERENCES

Andersen, N.M., Cheng, L., Damgaard, J. and Sperling, F.A.H. 2000. Mitochondrial DNA sequence variation and phylogeography of oceanic insects (Hemiptera: Gerridae: *Halobates* spp.). Marine Biology 136: 421-430.

Arnold, J. 1993. Cytonuclear disequilibria in hybrid zones. Annual Review of Ecology and Systematics 24: 521-554.

Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York, New York.

Asmussen, M.A., Arnold, J. and Avise, J.C. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. Genetics 115: 755-768.

Asmussen, M.A. and Basten, C.J. 1994. Sampling theory for cytonuclear disequilibria. Genetics 138: 1351-1363.

Aubert, J. and Solignac, M. 1990. Experimental evidence for mitochondrial DNA introgression between *Drosophila* species. Evolution 44: 1272-1282.

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York, New York.

Avise, J.C., Arnold, J., Ball, R.M., Eldredge, B., Lamb,T., Neigel, J.E., Reeb, C.A. and Saunders, N.C. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489-522.

Barton, N.H. and Hewitt, G.M. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113-48.

Brown, A.H.D. 1975. Sample sizes required to detect linkage disequilibrium between two or three loci. Theoretical Population Biology 8: 184-201.

Caterino, M.S. and Sperling, F.A.H. 1999. *Papilio* phylogeny based on mitchondrial cytochrome oxidae I and II genes. Molecular Phylogenetics and Evolution 8:1-16.

Cho, S., Mitchell, A., Regier, J.C., Mitter, C., Poole, R.W., Friedlander, T.P. and Zhao, S. 1995. A highly conserved nuclear gene for low-level phylogenetics:

Elongation Factor-1a recovers morphology-based tree for helopthine moths. Molecular Biology and Evolution 12: 650-656.

Clary, D.O. and Wolstenholme, D.R. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization, and genetic code. Journal of Molecular Evolution 22: 252-271.

Cooper, S.J.B. and Hewitt, G.M. 1993. Nuclear DNA sequence divergence between parapatric subspecies of the grasshopper *Chorthippus parallelus*. Insect Molecular Biology 2: 185-194.

Coyne, J.A. and Orr, H.A. 1989. Two rules of speciation. Pp 180-207 *in* D. Otte and J. Endler, eds., Speciation and its Consequences. Sinauer Associates, Sunderland, MA.

Crapon de Caprona, M.D. 1986. Are "preferences" and "tolerances" in cichlid mate choice important? Journal of Fish Biology 29: 151-158.

Damgaard, J. 1999. Molecular phylogenetics and genetic diversity of water striders (Heteroptera: Gerridae). PhD thesis, University of Copenhagen, Denmark.

Damgaard, J., Andersen, N.M. and Sperling, F.A.H. 2000. Phylogeny of the water strider genus *Aquarius* Schellenberg (Heteroptera: Gerridae) based on nuclear and mitochondrial DNA sequences and morphology. Insect Systematics and Evolution 31: 71-90.

Damgaard, J. and Sperling, F.A.H. 2001. Phylogeny of the water strider genus *Gerris* Fabricius (Heteroptera: Gerridae) based on CO1 mtDNA, EF1-α nuclear DNA and morphology. Systematic Entomology 26: 241-254.

Dobzhansky, T. 1951. Genetics and the Origin of Species, 3rd ed. Columbia University Press, New York.

Excoffier, L., Smouse, P., and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fairbairn, D.J. and Butler, T.C. 1990. Correlated traits for migration in the Gerridae (Hemiptera, Heteroptera): a field test. Ecological Entomology 15: 131-142.

Fritz, G.N., Conn, J. and Seawright, J. 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anopheles nuneztovari* (Diptera: Culicidae). Molecluar Biology and Evolution 11: 406-416.

Galacatos, K., Cognato, A.I. and Sperling, F.A.H. 2002. Population genetic structure of two water strider species in the Ecuadorian Amazon. Journal of Freshwater Biology (in press).

Grant, P.R. and Grant, B.R. 1997. Mating patterns of Darwin's finch hybrids determined by song and morphology. Biological Journal of the Linnean Society 60: 317-343.

Guo, S. and Thompson, E. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Haldane, J.B.S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12: 101-109.

Harrison, R.G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56: 337-349.

Harrison, R.G. 1993. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York, New York.

Harrison, R.G. and Bogdanowicz, S.M. 1997. Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. Evolution 51: 493-505.

Hewitt, G.M. 1993. After the ice: *parallelus* meets *erythropus* in the Pyrenees. Pp 140-164 *in* R.G. Harrison, ed. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York.

Hewitt, G.M., Nichols, R.A., Barton, N.H. 1987. Homogamy in a hybrid zone in the alpine grasshopper *Podisma pedestris*. Heredity 59: 457-466.

Honda, J.Y., Nakashima, Y., Yanase, T., Kawarabata, T., and Hirose, Y. 1998. Use of the internal transcribed spacer (ITS-1) region to infer *Orius* (Hemiptera: Anthocoridae) species phylogeny. Applied Entomology and Zoology 33: 567-571.

Hovemann, B., Richter, S., Walldorf, U. and Cziepluch, C. 1988. Two genes encode related cytoplasmic elongation factors 1 alpha (EF-1 alpha) in *Drosophila melanogaster* with continuous and stage specific expression. Nucleic Acids Research 16: 3175-3194.

Hillis, D.M. and Dixon, M.T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quarterly Review of Biology. 66: 411-453.

Hudson, R.R., Slatkin, M. and Maddison, W.P. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132: 583-589.

Hutter, C.M. and Rand, D.M. 1995. Competition between mitochondrial haplotypes in distinct nuclear genetic environments: *Drosophila pseudoobscura* vs. *D. persimilis*. Genetics 140: 537-548.

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

Kaneshiro, K.Y. and Giddings, L.V. 1987. The significance of asymmetrical sexual isolation and the formation of new species. Evolutionary Biology 21: 29-43.

Karl, S.A., Bowen, B.W. and Avise, J.C. 1995. Hybridization among the ancient mariners: characterization of marine turtle hybrids with molecular genetic analysis. Journal of Heredity 86: 262-268.

Kilpatrick, S.T. and Rand, D.M. 1995. Conditional hitchhiking of mitochondrial DNA: Frequency shifts of *Drosophila melanogaster* mtDNA variants depend on nuclear genetic background. Genetics 141: 1113-1124.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. Journal of Evolutionary Biology 13: 756-765.

Lamb, T. and Avise, J.C. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behaviour. Proceedings of the National Academy of Science U.S.A. 83: 2526-2530.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Maddison, W.P. and Maddison, D.R. 1992. MacClade: Analysis of phylogeny and character evolution. Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Mallet, J., McMillan, W.O., and Jiggins, C.D. 1998. Mimicry and warning colour at the boundary between races and species. Pp. 390-403 *in* D.J. Howard and S.H. Berlocher, eds. Endless Forms, Species and Speciation. Oxford University Press, New York.

Marcon, P.C.R.G., Taylor, D.B., Mason, C.E., Hellmich., R.L. and Siegfried, B.D. 1999. Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* (Hübner) Lepidoptera: Crambidae). Insect Molecular Biology 8: 213-221.

Pamilo, P. and Nei, M. 1998. Relationships between gene trees and species trees. Molecular Biology and Evolution 5: 568-583.

Paterson, H.E.H. 1993. Evolution and the Recognition Concept of Species: Collected writings. Johns Hopkins University Press, Baltimore.

Piertney, S.B., MacColl, A.D.C., Bacon, P.J., Racey, P.A., Lambin, X., and Dallas, J.F. 2000. Matrilineal genetic structure and female-mediated gene flow in red grouse (*Lagopus lagopus scoticus*): an analysis using mitochondial DNA. Evolution 54:279-289.

Prowell, D.P. 1998. Sex linkage and speciation in Lepidoptera. Pp. 309-319 *in* D.J. Howard and S.H. Berlocher, eds. Endless Forms, Species and Speciation. Oxford University Press, New York.

Rich, S.M., Rosenthal, B.M., Telford III, S.R., Speilman, A., Hartl, D.L. and Ayala, F.J. 1997. Heterogeneity of the internal transcribed spacer (ITS-2) region within individual deer ticks. Insect Molecular Biology 6: 123-129.

Ridely, M. 1993. Evolution. Blackwell Scientific Publications, Inc., Cambridge, Massachusetts.

Robertson, H.M. 1983. Mating behaviour and the evolution of *Drosophila mauritania*. Evolution 37: 1283-1293.

Shapiro, L.H. 1998. Hybridization and geographic variation in two meadow katydid contact zones. Evolution 52: 784-796.

Shoemaker, D.D., Ross. K.G. and Arnold, M.L. 1996. Genetic structure and evolution of a fire ant hybrid zone. Evolution 50: 1958-1976.

Simon, C., Frati, F., Beckenback, A., Crespi, B., Liu, H. and Flook, P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Annals of the Entomological Society of America 87: 651-701.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47: 264-279.

Slatkin, M. and Maddison, W.P. 1989. A cladistic measure of gene flow inferred from phylogeny of alleles. Genetics 123: 603-613.

Spence, J.R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Canadian Journal of Zoology 68: 1770-1782.

Spence, J.R. 2000. Seasonal aspects of flight in water striders (Hemiptera: Gerridae). Entomological Science 3: 399-417.

Spence, J.R. and Maddison, D.R. 1986. Chromosomes of two hybridizing species of *Limnoporus* (Heteroptera: Gerridae). Proceedings of the Entomological Society of Washington 88: 502-508.

Spence, J.R. and Wilcox, R.S. 1986. The mating system of two hybridizing species of water striders (Gerridae) II. Alternative tactics of males and females. Behavioural Ecology and Sociobiology 19: 87-95.

Sperling, F.A.H. 1987. Evolution of the *Papilio machaon* species group in western Canada. Quaestiones Entomologica 23: 198-315.

Sperling, F.A.H. 1993. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. Heredity 71: 227-233.

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

Sperling, F.A.H. 2002. Butterfly molecular systematics: from species definitions to higher level phylogenies. *In* C. Boggs, W. Watt, and P. Ehrlich, eds. Ecology and Evolution Taking Flight: Butterflies as Model Study Systems. University of Chicago Press, Chicago.

Sperling, F.A. H. and Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370-1383.

Sperling, F.A.H., Spence, J.R. and Andersen, N.M. 1997. Mitochondrial DNA, allozymes, morphology and hybrid compatability in *Limnoporus* water striders (Heteroptera: Gerridae): Do they all track species phylogenies? Annals of the Entomological Society of America 90: 401-415.

Syzmura, J.M. and Barton, N.H. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45: 237-261.

Tegelström, H. and Gelter, H.P. 1990. Haldane's rule and sex-biased gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). Evolution 44: 2012-2021.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. Pp. 3-27 *in* Speciation and its Consequences. D. Otte and J. A. Endler, eds. Sinauer Assocaites, Sunderland, MA.

Thulin, C.G., Jaarola, M. and Tegelström, H. 1997. The occurence of mountain hare mitochondrial DNA in wild brown hares. Molecular Ecology 6: 463-467.

Vazquez, P., Cooper, S.J.B., Gosalvez, J. and Hewitt, G.M. 1994. Nuclear DNA introgression across a Pyrenean hybrid zone between parapatric subspecies of the grasshopper *Chorthippus parallelus*. Heredity 73: 436-443.

Wang, R.L., Wakeley, J. and Hey, J. 1997. Gene flow and natural selection in the origin of *Drosophila pseudoobscura* and close relatives. Genetics 147: 1091-1106.

Wesson, D.M., Porter, C.H. and Collins, F.H. 1992. Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Molecular Phylogenetics and Evolution 1: 253-269.

Whitlock, M.C. and McCauley, D.E. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82: 117-125.

Wirtz, P. 1999. Mother species-father species: unidirectional hybridization in animals with female choice. Animal Behaviour 58: 1-12.

MORPHOMETRIC VARIATION AMONG HYBRIDIZING *LIMNOPORUS* WATER STRIDERS

INTRODUCTION

Identification of patterns of character expression in naturally generated hybrids is useful for conservation management of species that hybridize (Douglas et al. 1988, Muoneke and Maughan 1991), improves phylogenetic analyses (McDade 1990, Rieseberg and Ellstrand 1993), and is of theoretical importance to evolutionary biologists (Strauss 1986, Luebke et al. 1988). Yet fundamental problems persist: how can natural hybrids be identified phenotypically and how can the degree of hybridization affecting individuals be assessed? Before molecular techniques were widely used, construction of various hybrid indices based on morphology was the primary method for investigating patterns of differentiation within hybrid zones. Although molecular markers provide new insights into hybridization, morphological traits remain useful because they can be assessed in the field and have the potential to provide a simple basis for assessing the degree of hybridization. An obvious but seldom-met requirement for studies that effectively describe morphological variation between hybrids and parental species are data gathered from hybrids of known ancestry.

Methodological problems associated with describing patterns of variation in hybrids arise in the absence of a known hybrid sample (Adams 1982). Assumptions about the degree of variation within parental and hybrid populations all too often remain untested (Neff and Smith 1979). For example, suspected hybrids may be treated as a third a priori group for canonical variate analysis (Colgan et al. 1976). In some studies,

discriminant scores for hybrids have been calculated a posteriori from canonical coefficents that were generated using only the parental species (e.g. Lamb and Avise 1987, Crespin and Berrebe 1999). In these studies, scores for hybrids are assumed to fall somewhere between the two known groups. Furthermore, problems arise when laboratory-reared hybrids are included in an analysis but are compared to field-collected individuals representing the 'parental' species. In such studies, it is not clear if patterns of variation between hybrids and the parental species are due to the laboratory environment or represent a genetically based pattern of morphological variation (e.g. Leong and Hafernik 1992).

A common assumption is that individuals of unknown ancestry are hybrids if they display charactertistics intermediate to the presumed parental species (e.g. Strauss 1986), with a relatively high degree of variance (e.g. Hubbs 1955). However, in hybrid plants, morphological character expression is represented by both parental and intermediate characters rather than strictly intermediate ones (Rieseberg and Ellstrand 1993), nor are hybrid fish consistently more variable in any character (Neff and Smith 1979). The genetic basis of morphological differences may vary among taxa (Rieseberg and Ellstrand 1993), and thus characters selected for study need not always have patterns intermediate between the parental species (Warwick et al. 1992). Thus, the assumption of hybrid intermediacy should be empirically tested before using morphometrics to identify hybrids.

One way to quantify the degree of morphological variation between hybrid insects and their parents is to measure laboratory-reared specimens from known crosses.

Laboratory-reared hybrids of *Gerris* water striders were intermediate in size between the parental species (Largiader et al. 1994). Conversely, Leong and Hafernik (1992) found that F_1 hybrids of two damselfly species could not be distinguished from one of the parental species. With known hybrids, it may be possible to develop discriminant functions to classify unknown individuals from wild populations. For example, two subspecies of *Papilio* butterflies and their laboratory-reared F_1 hybrids can be classified with 84% accuracy using three wing characters (Luebke et al. 1988).

Two species of water striders, *Limnoporus notabilis* and *L. dissortis*, are distinguishable morphometrically and hybridize in the foothills of the Rocky Mountains in western Alberta and in central British Columbia (Spence 1990, Sperling and Spence 1991). Specimens from allopatric populations are easily distinguished by body size: *L. notabilis* are larger (male range: 15.5-19.2 mm, female range: 16.5-19.7 mm) than *L. dissortis* (male range: 11.5-15.0 mm, female range: 13.0-15.1) (Andersen and Spence 1992). In addition, several subtle structural traits further distinguish these species (see Andersen and Spence 1992). Presently, hybrids are indentified if their body length falls within a range (15.1-15.7 mm) intermediate to *L. notabilis* and *L. dissortis*. Only male hybrids are identified with this criterion because female F_1 hybrids are extremely difficult to produce in the laboratory (Spence 1990, Sperling et al. 1997), and are probably also rare in nature (Sperling and Spence 1991, see Chapter 2). However, morphological variation among F_1 hybrids, backcross hybrids and their parents has not been explicity examined. A strong association between allozyme markers and the genes affecting

morphometric variation (Klingenberg et al. 2000), suggests that morphology is a useful indication of genotype within the *Limnoporus* hybrid zone.

The purpose of this study is to examine morphometric variation in laboratory-reared *L. notabilis*, *L. dissortis* and their F_1 and backcrossed hybrids, to assess how well these groups can be distinguished using variable a priori group assignments in a multivariate discriminant analysis. The results support the intermediacy of hybrid morphology, but also demonstrate the limitations of distinguishing between F_1 and backcrossed hybrids on the basis of morphology alone.

METHODS

Laboratory cultures

Field-captured *L. notabilis* (from Lansdowne, B.C.) and *L. dissortis* (near Spruce Grove, Alberta) were reared in the laboratory for two and three generations, respectively, and the specimens were used as reference groups. F_1 males were produced from reciprocal crosses (*L. notabilis* x *L. dissortis* and *L. dissortis* x *L. notabilis*). Backcrossed individuals were produced from four subsequent crosses using the above parental stocks (females are always designated first in the cross): 1) *L. notabilis* x hybrid (*L. notabilis* x *L. dissortis*), 2) *L. notabilis* x hybrid (*L. dissortis* x *L. notabilis*), 3) *L. dissortis* x hybrid (*L. notabilis* x *L. dissortis*), and 4) *L. dissortis* x hybrid (*L. dissortis* x *L. notabilis*).

Water striders were reared in plastic tubs and fed freshly frozen flesh flies (*Sarcophaga bullata* Parker) ad libitum approximately every other day. They were maintained at a 19L:5D photoperiod and a temperature of either 24°C or 20°C. Sample

sizes by sex for all groups are shown in Table 3-1. A limited number of *L. notabilis* females (n=5) were available for measurement. Parental stocks were reared in the laboratory during April to July 1988. Hybrids were produced in the laboratory at various times from 1989 to 1992. Specimens, as enumerated in Table 3-1, were preserved in alcohol.

Seven morphometric traits were measured: total body length (from the tip of the head to the end of the abdomen, at the distal end of the proctiger) and lengths of mesofemur, mesotibia, metafemur, metatibia, first antennal segment and fourth antennal segment. I selected these traits because measurements of body size and leg lengths are relatively easy to measure and can be assessed in the field. Characters such as body length and lengths of mesofemur, mesotibia and fourth antennal segment distinguish the two parental species (Andersen and Spence 1992, Klingenberg et al. 2000). Although differences in leg lengths between *L. notabilis* and *L. dissortis* are allometric (Andersen and Spence 1992), patterns of correlation in hybrids can be quite different from that of either parent (Adams 1982).

Most measurements were made using a dissecting microscope fitted with a camera lucida and a digital measuring tablet. Some measurements of body length were made using only a dissecting microscope. Mid legs, hind legs and antennae were mounted with transparent tape onto glass slides before being measured.

Analysis

I used canonical variate analysis to examine morphometric differentiation among parental species and hybrids. Canonical variate analysis maximizes the differences

between predefined groups relative to variation within groups (Albrecht 1980, Campbell and Atchley 1981), providing a set of uncorrelated linear functions (canonical variates, CVs) that describe the best mathematical separation of the groups.

In a preliminary analysis, we included all 7 characters and analysed males and females separately. For males, *L. notabilis*, *L. dissortis*, each of the two reciprocal F_1 crosses and backcrosses to either maternal parent comprised six reference groups. A lack of F_1 hybrids in females resulted in only four reference groups (*L. notabilis*, *L. dissortis* and backcrossed hybrids to either maternal parent). In total, 103 males and 48 females were included in canonical variate analyses.

Effects of laboratory environment

I also compared variation between laboratory-reared and field-collected specimens of *L. dissortis* and *L. notabilis*. *L. notabilis* (males n=33, females n=47) were collected near Vancouver, B.C. at Malcolm Knapp Research Forest (University of British Columbia) and *L. dissortis* (males n=31, females n=47) were collected in eastern Alberta, south of Bonnyville. The assumption that populations at these sites were free from the influence of interspecific gene introgression was supported by the homozygous state of species-specific alleles at two nuclear loci and one mitochondrial locus described for 10 individuals within the same region (see Chapter 2). Morphological traits of field collected individuals were measured as previously described for laboratory-reared bugs.

I used canonical variate analysis to assess the degree of variation between laboratory-reared and field-captured *L. notabilis* and *L. dissortis*, analysed separately by

sex (four groups). Correspondence in patterns of variation between parental groups caught in the wild and raised in the laboratory was interpreted as genetically determined variation. Differences in patterns of variation between these groups was interpreted as non-genetic variation induced by the laboratory environment.

Identification in natural populations

Fisher's linear discriminant functions can be used directly in classification by assigning an unknown to the group for which it has the best fitting discriminant score. Using CVA, a discriminant score for each known group (*L. dissortis*, *L. notabilis* or hybrids) is obtained by multiplying the standardized coefficients by the values of the variables, summing these products, and adding the constant.

Stepwise discriminant function analysis can reduce the number of variables required to distinguish between groups. The elimination of unnecessary variables (i.e. those that do not improve separation between groups) is desirable because their inclusion may increase the number of misclassifications produced by discriminant functions (Klecka 1980). A forward selection method uses Wilk's lambda as selection criteria for adding variables one at a time. The first variable selected in a stepwise procedure accounts for most of the variance between groups and the variable that is selected next accounts for most of the remaining variation between groups. Variables are added until the selection of an additional variable does not signficantly increase group separation.

Differences in morphology between laboratory-raised and wild-caught *Limnoporus* (see Results) probably account for a high percentage of identification errors of wild-caught individuals in a preliminary test of discriminant functions that were

developed using laboratory-raised individuals. In an effort to decrease identification error, we ran another discriminant function analysis by pooling the laboratory-raised parental and field-collected individuals into one group for each species. We also pooled all hybrid types together (F_1s and backcrosses). The three known groups that were used in the analysis were *L. notabilis* (laboratory-reared and wild, males n=47, females n=52), *L. dissortis* (laboratory-reared and wild, males n=48, females n=61) and all F_1 hybrids and backcrossed individuals (laboratory-reared, males n=73, females n=29).

Testing the discriminant functions

I tested the discriminant functions (described above) using the same data set they were developed with, to assess how well they identified individuals that were not raised in the laboratory. The percentage of correctly identified individuals to be expected is probably overestimated because the discriminant functions were computed and tested on the same data set. Therefore, we also tested discriminant functions on natural populations not included in the original data set. We assumed that populations were either "pure" or hybridized on the basis of distance from the hybrid zone, and if they were located in regions that were previously shown to be extensively hybridized or not (Sperling and Spence 1991). Although I was not able to assess if unknown individuals were correctly identified, this procedure at least showed if hybrids were detectable in known hybrid populations and in populations where they were not likely to be found.

I selected natural populations that had not been included in the initial analysis, to represent each known group dealt with by the discriminant functions. *L. notabilis* populations were collected from three populations on the west side of the Rockies in

British Columbia, along Highway 23, between the Selkirk mountains to the east and the Monashee mountains to the west. Adjacent populations were separated by 6 km and 78 km. Sample sizes ranged from 15-20 individuals with a mean sample size of 17.3 individuals. *L. dissortis* were collected from 3 populations in eastern Alberta, east of Edmonton. Sample sizes ranged from 10-30 individuals (mean=19.3). To represent hybrid populations, I selected populations along the eastern foothills of the Rockies, in southern Alberta where hybridization is extensive (Sperling and Spence 1991). Sample sizes ranged from 6-17 individuals (mean=11.3), and adjacent populations were separated by 31 km and 130 km.

RESULTS

Laboratory-reared males

In a canonical variate analysis using six reference groups, *L. notabilis* and *L. dissortis* males were well separated along the first canonical variate (CV1), with hybrids and backcrosses somewhat intermediate and further separated from the parentals along CV2 (Figure 3-1a). Reciprocal F_1 hybrids overlap in multivariate space, but backcrosses to either maternal parent are somewhat separated along CV1. Most of the variation was explained by the first 2 canonical variates; CV1 and CV2 explained 68.2% and 23.4% of the variability among reference groups, respectively. The standardized canonical vector coefficients indicate the relative importance of each character in distinguishing between groups (Table 3-2a), although direct interpretations are complicated by intercorrelations among characters and differing variances of the characters (Klingenberg 1992).

Nonetheless, mesotibia and metatibia measurements contributed the most to distinguishing the groups. Interestingly, body length is relatively unimportant for distinguishing male groups along CV1. Most hybrids cluster on the lower half of CV2. Position along CV2 is determined mainly by body length and mesofemur (Table 3-2a). When tested against the original data, the percentage of correct classifications for *L. notabilis* was 92.9%, *L. dissortis* 87.5%, F_1 (*L. notabilis* x *L. dissortis*) 45.5%, F_1 (*L. dissortis* x *L. notabilis*) 75.0%, B_1 (*L. notabilis* mother) 56.7% and B_1 (*L. dissortis* mother) 45.8%.

Laboratory-reared females

Females are also well separated along the CV1 axis, which explains about the same amount of variation as for males. CV1 explains 60.4% of the variation between groups and 27.5% is explained by CV2 (Figure 3-1b). Separation along CV1 is mostly determined by metatibia, mesotibia and mesofemur (Table 3-2b). As for males, body length is relatively unimportant for distinguishing female groups along CV1. Female hybrids and parental species are somewhat separated on CV2, with hybrids in this case mainly along the upper half of the CV2 axis. When compared to the CVA plot for males, the orientation of CV2 for females is flipped. Separation of female backcross progeny by the maternal parent are not as apparent as for males, because backcrosses to either a *L. dissortis* or *L. notabilis* mother are not distinguished on a CVA plot (Figure 3-1b). Mesofemur and body length contribute the most to distinguishing groups along CV2

(Table 2b). The percentage of correct classifications for *L. notabilis* was 100%, *L. dissortis* 85.7%, B_1 (*L. notabilis* mother) 57.1% and B_1 (*L. dissortis* mother) 75.0%.

Comparison of laboratory-reared and natural parental populations

Patterns of variation among populations raised in the laboratory correspond to patterns of variation among populations collected from natural populations. For both sexes, CV1 separates the parental species, demonstrating the primary importance of the genetic basis of morphometric variation (Figure 3-2a, 3-2b); in fact, it explains most of the variation between reference groups for males (92.2%) and females (94.3%).

CV2 mainly distinguished laboratory-reared and wild-caught individuals *of L. notabilis* for both sexes. For males of both species, individuals from laboratory cultures tend to be positioned higher on the CV2 axis than the wild individuals but the difference between laboratory and field cultures is greater for *L. notabilis* than *L. dissortis (*Figure 3-2a*)*. CV2 explains 7.3% of the variance between male groups and is mostly determined by metatibia, body length, mesotibia and mesofemur (Table 3-3a). For females, laboratory-reared individuals appear lower along the CV2 axis than the field-caught ones. As in males, the difference between laboratory-raised and wild-caught females is greater for *L. notabilis* relative to *L. dissortis* (Figure 3-2b). CV2 accounts for 4% of the variation between groups, and is mostly distinguished by body length (Table 3-3b).

I analysed species individually using CVA to assess which traits contribute the most to differences between laboratory-raised and wild-caught individuals. For *L. notabilis* of both sexes, lengths of mesofemur and metatibia distinguished laboratory-raised and wild-caught individuals (Table 3-4a), with longer legs generally observed in

wild-caught individuals (Table 3-5a). However, body length and the length of the first antennal segment are longer in laboratory-raised females; the opposite is true for males (Table 3-5a). These variables are more useful for discriminating between specimens from the laboratory or field for female *L. notabilis* than for males (Table 3-4a).

For *L. dissortis*, interpreting patterns of variation for both sexes was not as clear cut. Some of the variables that were most important for distinguishing between laboratory-reared or wild-caught males (e.g., meso and metatibiae) and females (e.g. mesofemur) were not important for the other sex (Table 3-4b). Although metafemur length was the most important variable for both sexes, it tended to be larger in wild-caught males compared to laboratory-reared males whereas in females, the opposite was true (Table 3-5b). Body length was also a relatively good discriminator between groups for both *L. dissortis* sexes, with laboratory-reared individuals tending to have greater body lengths compared to wild-caught individuals (Table 3-5b).

Classification success

The apparent differences between laboratory-reared and natural parental populations (particulary of *L. notabilis*) and the poor resolution between male backcrossed and F_1 reciprocal crosses present challenges to the accurate classification of these groups. Thus, we made several changes to the discriminant function analysis to improve classification success in natural populations: 1) All hybrids were pooled into one reference group, 2) Laboratory-reared and field collected individuals were pooled for each parental species, 3) Stepwise discriminant function analysis was used to reduce the number of characters needed to distinguish between groups.

For males, stepwise discriminant analysis selected a reduced set of 3 variables. Body length and the lengths of the meso and metatibiae explained more variation between 3 reference groups than was explained for the original data set. CV1 and CV2 explained 97.5% and 2.5% of the variation, respectively (Figure 3-3a). Separation of parental species and hybrids was along CV1 and mostly determined by mesotibial length (Table 3-6a). Groups were not separated along CV2. The percentage of corrrect classifications also was improved over the preliminary analysis as correct identification was achieved for 100% of *L. notabilis*, 97.9% of *L. dissortis* and 82.2% of hybrids.

For females, only 2 variables, the length of meso and metatibiae, were selected from the full set using stepwise discriminant analysis. Mesotibial length contributed the most to separation along the CV1 axis, which explained 95.6% of the variation between groups (Table 3-6b). CV2 explained 4.4% of the variation. There is some separation of hybrids along CV2, although the parental species are less separated along the CV1 axis in females compared to males (Figure 3-3b), resulting in more overlap between hybrids and the parental species. Consequently, the percentage of correct classifications was high for the parental species (*L.notabilis* 90.4%, *L. dissortis* 83.6%) but low for backcrossed hybrids (55.2%).

Testing discriminant functions in natural populations

Wild-caught males

When we applied the linear discriminant functions derived from stepwise analysis with three known groups (Table 3-7) to natural populations not included in the original data set, we found that the classifications produced by the discriminant functions

coincided well with our expectations based on previous studies (Table 3-9). Within *L. notabilis* populations, 90.4% of individuals were classified as *L. notabilis* and 9.6% were classified as hybrids. Within *L. dissortis* populations, 98.3% were classified as *L. dissortis* and 1.7% were classified as hybrids. In populations from the eastern slopes of the Rockies, where I expected to find hybrids, the functions developed classified 29.4% of individuals as hybrids, 29.4% *L. notabilis* and 41.2% *L. dissortis*. Thus, for males, the discriminant functions are able to detect hybrids in naturally hybridized populations. However, they also may classify up to 10% of individuals in presumably "pure" populations as hybrids. Misidentifications of a hybrid as a a parental species may contribute to a relatively greater proportion of error, if most hybrid males in wild populations result from backcrossing.

Wild-caught females

We applied the linear discriminant functions derived from stepwise analysis with three known groups (Table 3-8) to females from wild populations not included in the original data set (Table 3-9). The number of probable misclassifications in wild populations of *L. notabilis and L. dissortis* were similar to each other; in the *L. notabilis* populations, 86.5% of individuals were classified as *L. notabilis,* and 13.5% were classified as hybrids. By comparison, in *L. dissortis* populations from eastern Alberta, 87.0% of individuals were classified as *L. dissortis* and 13% classified as hybrids. Hybridized populations sampled from the eastern foothills were characterized by 34.1% *L. notabilis*, 36.6% *L. dissortis* and 29.3% hybrids. Interestingly, the number of hybrids identified from presumably hybridized populations were similar for both males (29.4%)

and females (29.3%). The number of "pure" females misclassified as a hybrid may be as high as 13.5%, slightly higher than the number of misclassifications for males.

Morphometric characters are a useful tool for identifying *Limnoporus* species composition of field populations. The main advantage of using morphometric characters is that they can be used to identify hybrid populations relatively quickly. Thus, a preliminary description of regional patterns of hybridization provides a sound basis for testing process-oriented hypotheses.

DISCUSSION

Hybrid males are distinguishable from those of either *L. notabilis* or *L. dissortis*, based on 3 morphological measurements. However, the morphometric approaches used here could not correctly classify a high percentage of laboratory-reared female backcrossed hybrids, nor could they reliably distinguish between backcrossed males and F_1 hybrids. Although the discriminant functions are limited in their ability to distinguish individuals in this way, they are useful for estimating the extent of hybridization within populations.

It is surprising that the number of hybrids in wild, mixed populations was similar for both sexes because the classification success for hybrids was low in a previous test on laboratory-reared individuals. However, absence of F_1 females from the laboratory cultures probably accounts for the low classification success in the previous test of the discriminant functions. A similar proportion of classified hybrids for both males and females suggests that hybrids in the wild populations we tested are probably

backcrossed, and are therefore more likely to be mistakenly classified as a parental species.

Because backcrossed individuals carry a greater genetic contribution from one of the parental species, it is not surprising that some backcrosses are difficult to distinguish morphologically from one parental species. Lamb and Avise (1987) also had difficultly in distinguishing among backcrossed individuals, F_1 hybrids and parental species of hybridizing tree frogs. They found that hybrids backcrossed to both species were misclassified as a parental species at least 50% of the time and that 27% of backcrossed hybrids were misclassified as F_1 hybrids. Similarly, for hybridizing plant species in the genus *Carduus* there is considerable morphological overlap of both backcrosses and hybrids with parental plants as well as with each other (Warwick et al. 1992). The consequences of misidentifying backcrossed hybrids will vary with the relative abundance of F_1 and backcrossed hybrids within the hybrid zone. For example, F_1 hybrids are rare or absent in some hybrid zones yet must occur occasionally because recombinant genotypes are relatively common (Cruzan and Arnold 1993, Syzmura and Barton 1991). On the other hand, half of the hybrids are F_1 in a hybrid zone between *Heliconius* butterflies (Jiggins et al.1997).

Numbers of backcrossed hybrids within naturally hybridized populations are probably underestimated using the discriminant functions developed here, and estimates are more likely to be biased towards *L. dissortis*. Asymmetry in introgression demonstrates that natural hybridization does not equally affect *L. notabilis* and *L. dissortis* (Sperling and Spence 1991). Behavioural (Spence and Wilcox 1986) and genetic

factors (see Chapter 1) probably act to favour introgression of *L. dissortis* alleles into *L. notabilis* populations, rather than the reverse situation.

The limitations of using females to estimate the extent of hybridization is further emphasized here, because most female hybrids result from backcrossing (Spence 1990) and are therefore more likely to be misidentified. For males, comparisons of progeny backcrossed to either a *L. dissortis* or *L. notabilis* female suggest that backcrosses to an *L. dissortis* female are more likely to be mistaken as the maternal parent than are backcrosses to an *L. notabilis* female (see Figure 3-1a). However, a similar number of estimated hybrids in wild populations for both males and females suggests in some wild populations, this limitation may apply equally to both sexes. Although the proportion of backcrossed and F_1 hybrids may vary regionally, the results suggest hybrids in south-western Alberta result mainly from backcrossing. However, even when relatively few F_1 hybrids are produced, hybridized populations could arise through dispersal and breeding of the F_1 progeny. Applying the discriminant functions to other regions in the contact zone may not produce similar estimates for males and females if F_1 males are prevalent.

Variation in morphological traits between F_1 and backcrossed hybrids is consistent with a maternal influence on morphology. Reciprocal size differences in F_1 hybrids of *Melanoplus* grasshoppers (Chapco 1987), *Chorthippus* grasshoppers (Butlin and Hewitt 1987) and deer mice (Dawson et al. 1993), suggest the presence of either maternal effects or sex-linkage. Under a hypothesis of sex-linkage, we expect reciprocal F_1 differences to be observed in the heterogametic sex and not the homogametic sex. Maternal effects on growth can be detected if backcrossed progeny that differ only by the maternal parents

show differences in size. In *Limnoporus*, males are the heterogametic sex and do not show signs of reciprocal differences in F_1 crosses but backcrosses to either maternal parent do vary in multivariate space. These results suggest a role of maternal cytoplasmic factors on growth in *Limnoporus*.

When laboratory-raised hyrids are compared to field-collected parental species, differences in morphological variation may occur. This could be due to the increased food availability in the laboratory (e.g. Largiader et al. 1994). For *Gerris buenoi*, additional food increased the adult weight (Spence 1986) and size (Klingenberg and Spence 1997) under field conditions. Luebke et al. (1988) addressed this problem by feeding F_1 hybrids food of different qualities to encompass the natural variation in size caused by diet. In this study, most of the laboratory-raised water striders (except *L. notabilis* males) had a larger body size compared to wild-caught ones, and this probably resulted from increased food availability in the laboratory. We found that the influence of the laboratory environment on morphometric variation was greater for *L. notabilis* than *L. dissortis*.

Longer leg measurements for field-caught *L. notabilis* males and females compared to individuals raised in the laboratory is difficult to account for. The difference may be accounted by intraspecific variation among populations since individuals used to originate laboratory stocks and represent wild-caught populations came from different localities. Fairbairn (1984) reported significant, genetically based differences in *L. notabilis* body size between habitats at different elevations and suggested that selection on body size is influenced by the stability of habitats. If this is true, then the degree of variation between laboratory-raised and field-collected individuals could vary with the genetic stock of the

population the individuals were originally collected from. Increasing the sample sizes for field and laboratory-raised populations, and including individuals caught from different habitat types may better sample the range of variation within natural populations.

The observed effect of laboratory-rearing on morphological variation for *L. dissortis* and *L. notabilis* may include a component properly attributed to the time gap between establishing laboratory cultures and collecting individuals from natural populations. Individuals were reared in the laboratory and preserved in alcohol as early as 1989, 10 years prior to when individuals representing natural populations were collected. Klingenberg et al. (2000) documented changes in some regions of the hybrid zone from morphometric comparisons of samples collected in the 1980s and 1990s. Thus, it is possible that differences between laboratory and field-caught "pure" *Limnoporus* represent real changes, induced by introgression, in the morphology of individuals. However, evidence from two nuclear loci and one mitochondrial locus (see Chapter 2) from the Vancouver, British Columbia area suggest that *L. notabilis* measured from natural populations do not contain introgressed *L. dissortis* genes. Similarly, individuals measured from natural *L. dissortis* populations came from populations free of introgressed *L. notabilis* genes in eastern Alberta (see Chapter 2). Changes to the genetic composition of *L. dissortis* populations in eastern Alberta are extremely unlikely, given distance to the hybrid zone and the apparent westward movement of the zone. It is possible that some backcrossed specimens can be misidentified as a parental species even if they are monomorphic for species-diagnostic alleles at a locus (Lamb and Avise 1987).

The probability of committing this type of error decreases if more loci are used to classify individuals.

These results highlight the importance of including both molecular and morphological evidence when describing patterns of variation within hybrid zones. Although morphological evidence for hybridization is useful for finding populations where hybridization is most extensive, molecular tools are desirable to fully appreciate the extent and nature of gene exchange between two species. The ability to assay characters that are heritable and have a known mode of transmission is an advantage of genetic characters over most morphological ones, which may be influenced by non-genetic factors or have an unspecified genetic basis (Avise 1994). In particular, morphology alone will underestimate the extent of hybridization in hybrid zones where the majority of hybrids result from backcrossing due to their partial misidentification as parental stocks.

Table 3-1: Sample sizes of measured individuals from laboratory cultures of *L. notabilis*, *L. dissortis* and their hybrids

Genotype	males (n)	females (n)
L. notabilis	14	5
L. dissortis	16	14
F_1 (*L. notabilis* x *L. dissortis*)	11	0
F_1 (*L. dissortis* x *L. notabilis*)	8	0
B_1 (*L. notabilis* mother)	30	21
B_1 (*L. dissortis* mother)	24	8

Table 3-2a: Standardized canonical discriminant function coefficients (males). Analysis with 6 known laboratory-reared groups

Variable	Standardized coefficents for canonical variates	
	1	2
body length	0.655	1.814
mesofemur	0.207	-1.069
mesotibia	1.033	-0.423
metafemur	-0.491	-0.402
metatibia	-1.184	-0.607
1st antennal segment	0.669	0.487
4th antennal segment	-0.116	0.462

Table 3-2b: Standardized canonical discriminant function coefficients (females). Analysis with 4 known laboratory-reared groups

Variable	Standardized coefficents for canonical variates	
	1	2
body length	0.687	-1.649
mesofemur	-1.132	2.404
mesotibia	1.492	-0.188
metafemur	0.864	-0.561
metatibia	-1.311	-0.775
1st antennal segment	-0.024	0.869
4th antennal segment	-0.053	0.463

Table 3-3a: Standardized canonical discriminant function coefficients (males)
Analysis with 4 known groups: field captured and laboratory-reared
L. notabilis and *L. dissortis*

Variable	Standardized coefficents for canonical variates	
	1	2
body length	0.306	1.126
mesofemur	0.354	-1.004
mesotibia	1.813	1.056
metafemur	-0.534	-0.317
metatibia	-1.010	-1.371
1st antennal segment	-0.045	0.252
4th antennal segment	-0.325	0.494

Table 3-3b: Standardized canonical discriminant function coefficients (females)
Analysis with 4 known groups: field-captured and laboratory-reared
L. notabilis and *L. dissortis*

Variable	Standardized coefficents for canonical variates	
	1	2
body length	-0.108	-1.522
mesofemur	0.628	0.071
mesotibia	1.035	0.262
metafemur	-0.665	0.673
metatibia	-0.115	0.519
1st antennal segment	0.267	-0.118
4th antennal segment	-0.184	0.153

Table 3-4a: Standardized canonical discriminant function coefficients from 2 separate CVA analyses describing variation between 2 known groups: a) laboratory-raised and wild-caught *L. notabilis* males b) laboratory-raised and wild-caught *L. notabilis* females

Variable	**Standardized coefficents for canonical variates**	
	a) MALES	b) FEMALES
	1	1
body length	-0.722	-1.124
mesofemur	1.916	1.482
mesotibia	-0.634	0.699
metafemur	-0.365	-0.582
metatibia	1.107	1.107
1st antennal segment	-0.869	-1.359
4th antennal segment	-0.292	-0.223

Table 3-4b: Standardized canonical discriminant function coefficients from 2 separate CVA analyses describing variation between 2 known groups: a) laboratory-raised and wild-caught *L. dissortis* males b) laboratory-raised and wild-caught *L. dissortis* females

Variable	**Standardized coefficents for canonical variates**	
	a) MALES	b) FEMALES
	1	1
body length	1.409	0.765
mesofemur	0.288	1.625
mesotibia	1.503	-0.274
metafemur	-1.907	-2.127
metatibia	-1.264	0.684
1st antennal segment	-0.025	0.368
4th antennal segment	0.615	-0.259

Table 3-5a: Comparisons of mean +/- one standard deviation (S.D.) in millimetres, of measured variables for *L. notabilis* that were either raised in the laboratory or caught in natural populations. M= males, F= females

Variable	**Laboratory-reared** Mean +/- S.D. (mm) **M, n=14**	**Wild-caught** Mean +/- S.D. (mm) **M, n=33**	**Laboratory-reared** Mean +/- S.D. (mm) **F, n=5**	**Wild-caught** Mean +/- S.D. (mm) **F, n=47**
body length	17.98 +/- 0.66	18.15 +/- 0.69	17.88 +/- 0.97	17.51 +/- 0.59
mesofemur	11.64 +/- 0.54	13.05 +/- 0.82	10.03 +/- 0.62	10.57 +/- 0.47
mesotibia	9.67 +/- 0.50	10.68 +/- 0.63	8.25 +/- 0.51	8.67 +/- 0.38
metafemur	14.26 +/- 0.70	15.97 +/- 1.07	11.64 +/- 0.82	12.31 +/- 0.70
metatibia	8.85 +/- 0.64	10.27 +/- 0.69	7.63 +/- 0.81	8.32 +/- 0.46
1st antennal segment	3.13 +/- 0.15	3.30 +/- 0.21	2.71 +/- 0.22	2.61 +/- 0.15
4th antennal segment	1.84 +/- 0.10	1.81 +/- 0.09	1.71 +/- 0.06	1.72 +/- 0.06

Table 3-5b: Comparisons of mean +/- one standard deviation (S.D.) in millimetres, of measured variables for *L. dissortis* that were either raised in the laboratory or caught in natural populations. M= males, F= females

Variable	Laboratory-reared Mean +/- S.D. (mm) **M, n= 17**	Wild-caught Mean +/- S.D. (mm) **M, n=31**	Laboratory-reared Mean +/- S.D. (mm) **F, n=14**	Wild-caught Mean +/- S.D. (mm) **F, n=47**
body length	14.27 +/- 0.78	13.63 +/- 0.71	15.53 +/- 0.80	14.75 +/- 0.79
mesofemur	8.67 +/- 0.59	8.69 +/- 0.46	8.67 +/- 0.57	8.23 +/- 0.46
mesotibia	7.06 +/- 0.44	6.92 +/- 0.39	6.94 +/- 0.32	6.62 +/- 0.36
metafemur	10.68 +/- 0.79	10.79 +/- 0.59	10.00 +/- 0.48	9.72 +/- 0.58
metatibia	6.91 +/- 0.61	6.85 +/- 0.63	7.05 +/- 0.58	6.65 +/- 0.47
1st antennal segment	2.29 +/- 0.62	2.35 +/- 0.12	2.06 +/- 0.60	2.06 +/- 0.15
4th antennal segment	1.85 +/- 0.19	1.86 +/- 0.08	1.76 +/- 0.51	1.85 +/- 0.06

Table 3-6a: Standardized canonical discriminant function coefficients (males)
Stepwise analysis with 3 known groups: field collected and laboratory-reared parents and laboratory-reared hybrids

Variable	Standardized coefficents for canonical variates	
	1	2
body length	0.598	0.522
mesotibia	1.252	-2.167
metatibia	-0.92	2.154

Table 3-6b: Standardized canonical discriminant function coefficients (females)
Stepwise analysis with 3 known groups: field and laboratory-reared parentals and laboratory-reared hybrids

Variable	Standardized coefficents for canonical variates	
	1	2
mesotibia	1.291	-1.057
metatibia	-0.399	1.62

Table 3-7: Linear discriminant functions for males. Classification coefficents can be used directly for classification by multiplying by the variables, summing these products and adding the constant. An unknown case is assigned to the group for which it has the largest discriminant score.

Variable	Classification function coefficients per group		
	L. dissortis	*L. notabilis*	hybrids
body length	25.420	29.589	26.885
mesotibia	2.225	13.130	9.492
metatibia	-10.010	-16.997	-15.169
Constant	-150.585	-253.395	-189.917

Table 3-8: Linear discriminant functions for females. See Table 7 for interpretation.

Variable	Classification function coefficients per group		
	L. dissortis	*L. notabilis*	hybrids
mesotibia	30.561	43.017	37.405
metatibia	1.287	-2.050	-2.901
Constant	-107.755	-178.158	-127.139

Table 3-9: Classification of wild-caught individuals using discriminant functions derived from a stepwise analysis. LN= *L. notabilis*, LD= *L.dissortis*

		MALES				FEMALES			
Wild populations	**Location**	**n**	**LN**	**LD**	**hybrid**	**n**	**LN**	**LD**	**hybrid**
L. notabilis	Hwy. 23, about 56 km north of Revelstoke, B.C.	20	19	0	1	15	13	0	2
	Hwy. 23, about 7 km south of Mica Dam, B.C.	15	13	0	2	17	13	0	4
	Hwy. 23, about 62 km north of Revelstoke, B.C.	17	15	0	2	20	19	0	1
	TOTALS	**52**	**47**	**0**	**5**	**52**	**45**	**0**	**7**
	% OF TOTAL		**90.4%**	**0**	**9.6%**		**86.5%**	**0**	**13.5%**
mixed	Hwy 22, 25 km north of junction 541, west of Black Diamond	6	2	2	2	7	4	0	3
	Hwy 762, 12 km north of junction 549, south of Bragg Creek	17	5	7	5	24	5	10	9
	Hwy 40, 15 km north of hwy. 1A junction, near Cochrane	11	3	5	3	10	5	5	0
	TOTALS	**34**	**10**	**14**	**10**	**41**	**14**	**15**	**12**
	% OF TOTAL		**29.4%**	**41.2%**	**29.4%**		**34.1%**	**36.6%**	**29.3%**
L. dissortis	Hwy 36, south of Two Hills, eastern AB	10	0	10	0	12	0	11	1
	Hwy 36, south of Two Hills, eastern AB	18	0	18	0	26	0	22	4
	Hwy 881, north of St. Paul, eastern AB	30	0	29	1	39	0	34	5
	TOTALS	**58**	**0**	**57**	**1**	**77**	**0**	**67**	**10**
	% OF TOTAL		**0**	**98.3%**	**1.7%**		**0**	**87.0%**	**13.0%**

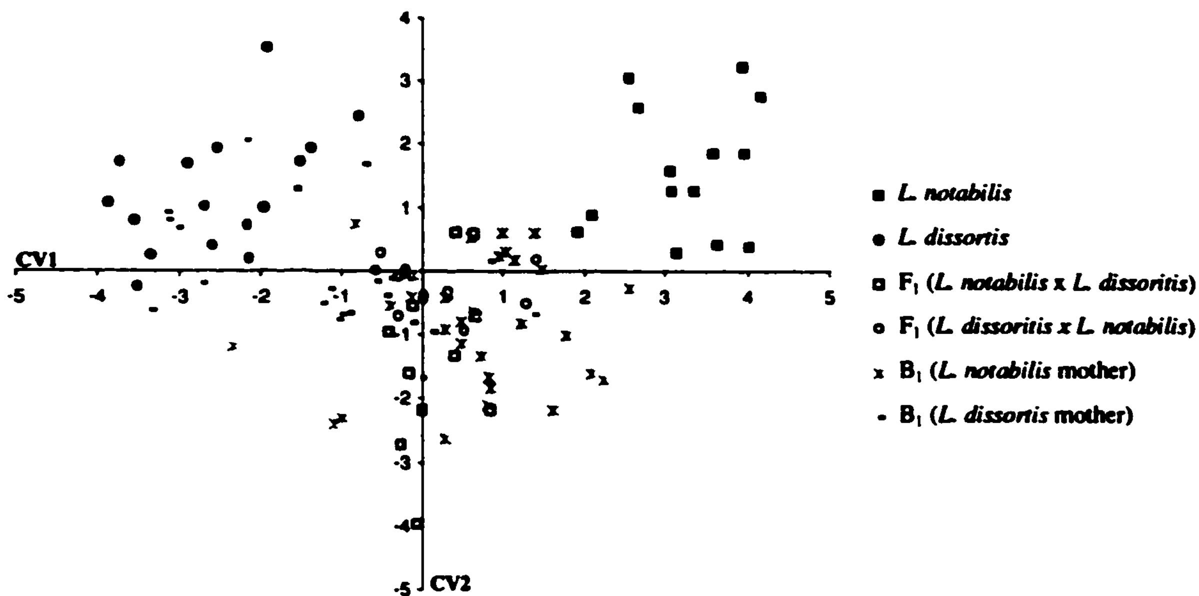

Figure 3-1a: Plot of CV1 and CV2 scores for laboratory-reared males (6 group discriminant analysis)

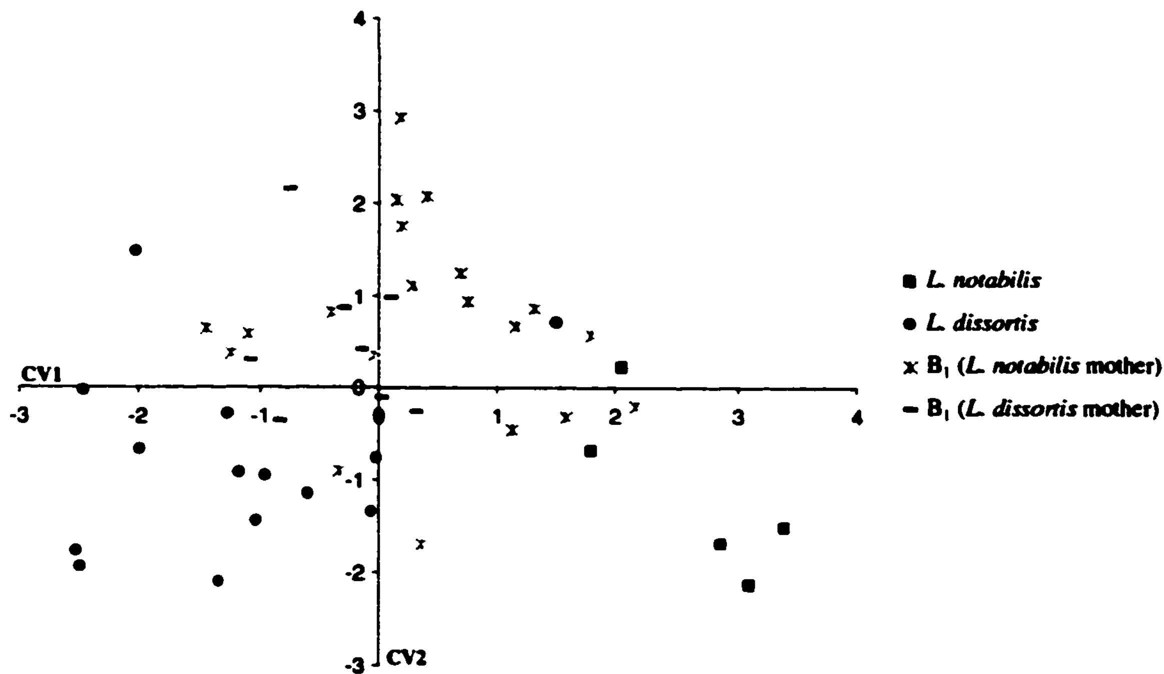

Figure 3-1b: Plot of CV1 and CV2 scores for laboratory-reared females (4 group discriminant analysis)

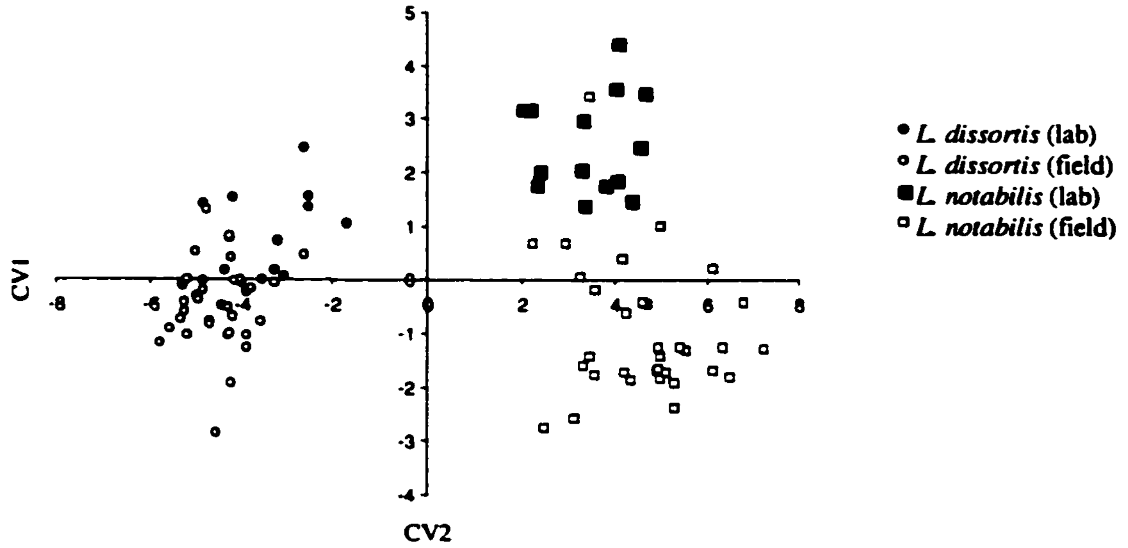

Figure 3-2a: Plot of CV1 and CV2 scores for male *L. notabilis* and *L. dissortis* that were laboratory-reared or field-caught.

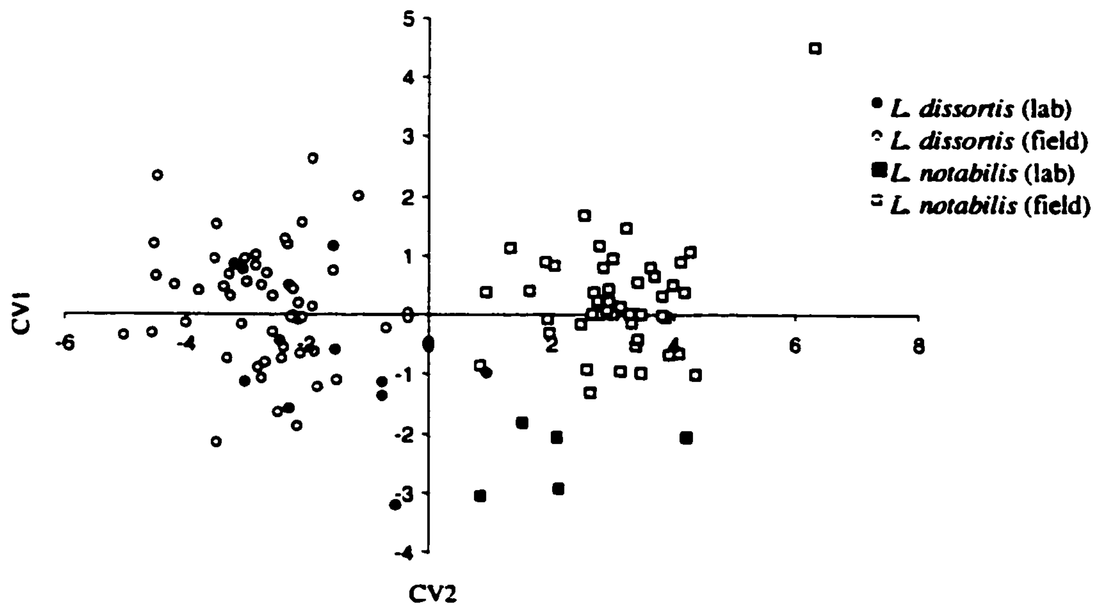

Figure 3-2b: Plot of CV1 and CV2 scores for female *L. notabilis* and *L. dissortis* that were laboratory-reared or field-caught.

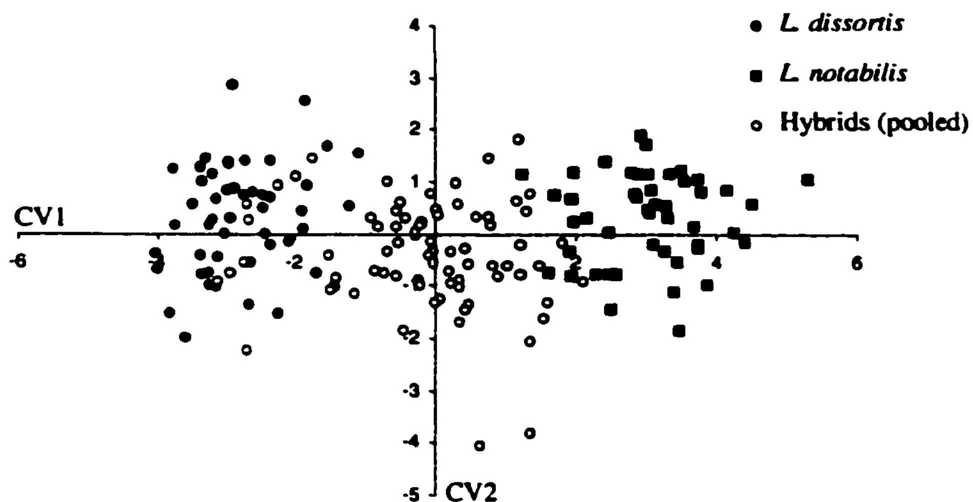

Figure 3-3a: CV1 and CV2 scores for males in a stepwise discriminant function analysis (3 groups). Parental species include individuals collected from the field and reared in the laboratory.

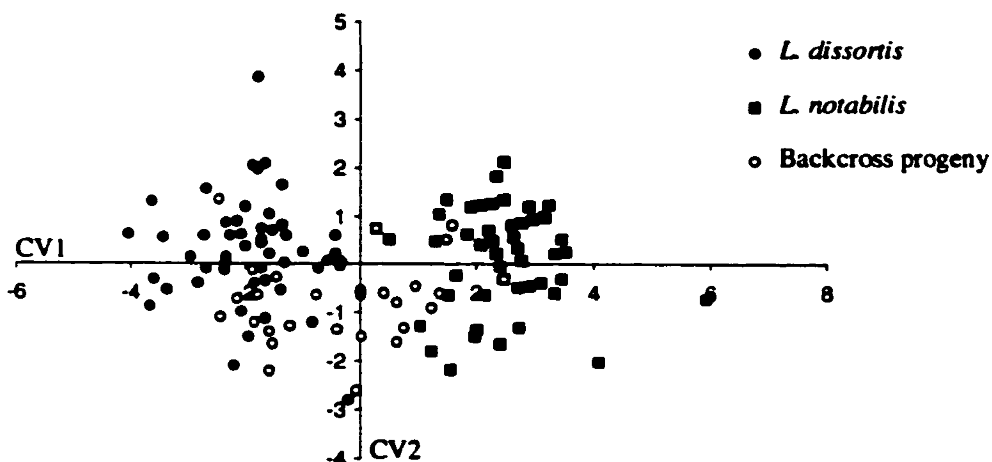

Figure 3-3b: CV1 and CV2 scores for females in a stepwise discriminant function analysis (3 groups). Parental species include individuals collected from the field and reared in the laboratory.

REFERENCES

Adams, R.P. 1982. A comparison of multivariate methods for the detection of hybridization. Taxon 31: 646-661.

Albrecht, G.H. 1980. Multivariate analysis and the study of form, with special reference to canonical variate analysis. American Zoologist 20: 679-693.

Andersen, N.M. and Spence, J.R. 1992. Classification and phylogeny of the Holarctic water strider genus *Limnoporus* Stål (Hemiptera, Gerridae). Canadian Journal of Zoology 70: 753-785.

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York, NY.

Butlin, R.K. and Hewitt, G.M. 1988. Genetics of behavioural and morphological differences between parapatric susbspecies of *Chorthippus parallelus* (Orthoptera: Acrididae). Biological Journal of the Linnean Society 33: 233-248.

Campbell, N.A. and Atchley, W.R. 1981. The geometry of canonical variate analysis. Systematic Zoology 30: 268-280.

Chapco, W. 1987. Genetic variation in three subspecies of *Melanoplus sanguinipes* (F.). I. Morphometric traits. Genome 29: 793-801.

Colgan, P.W., Ballantyne, P.K. and Wilson, M.V.H. 1976. Numerical taxonomy o hybridizing *Lepomis* (Centrarchidae) in Lake Opinicon, Ontario. Canadian Journal of Zoology 54: 42-47.

Crespin, L and Berrebi, P. 1999. Asymmetrical introgression in a freshwater fish hybrid zone as revealed by a morphological index of hybridization. Biological Journal of the Linnean Society 67: 57-72.

Cruzan, M.B. and Arnold, M.L. 1993. Ecologial and genetic associations in an *Iris* hybrid zone. Evolution 47: 805-8296.

Dawson, W.D., Sagedy, M.N., En-yu, L., Kass, D.H., and Crossland, J.P. 1993. Growth regulation in *Peromyscus* species hybrids: a test for mitochondrial-nuclear genomic interaction. Growth, Development and Aging 57: 121-133.

Douglas, M.E., Miller, R.R. and Minckley, W.L. 1988. Multivariate discrimination of Colorado Plateau *Gila* spp: the "art of seeing well" revisited. Transactions of the American Fisheries Society 127: 163-173.

Fairbairn, D.J. 1984. Microgeographic variation in body size and development time in the water strider, *Limnoporus notabilis*. Oecologica. 64: 126-133.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4: 1-20.

Jiggins, C.D., McMillan, W.O., King, P. and Mallet, J. 1997. The maintenance of species differences across a *Heliconius* hybrid zone. Heredity 79: 495-505.

Klecka, W.R. 1980. Discriminant analysis. Sage University Paper series. Quantitative Applications in the Social Sciences, Series No. 07-019. Beverly Hills and London: Sage Publications.

Klingenberg, C.P. 1992. Multivariate morphometrics of geographic variation of *Gerris costae* (Heteroptera: Gerridae) in Europe. Revue Suisse de Zoologie 99: 11-30.

Klingenberg, C.P. and Spence, J.R. 1997. On the role of body size for life-history evolution. Ecological Entomology 22: 55-68.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. Journal of Evolutionary Biology 13: 756-765.

Lamb, T. and Avise, J.C. 1987. Morphological variability in genetically defined categories of anuran hybrids. Evolution 41(1): 157-165.

Largiader, C.R., Klingenberg, C.P. and Zimmerman, M. 1994. Morphometric variation in a hybrid zone of two subspecies of *Gerris costae* (Heteroptera: Gerridae) in the Maritime Alps. Journal of Evolutionary Biology 7: 697-712.

Leong, J.M. and Hafernik Jr., J.E. 1992. Hybridization between two damselfly species (Odonata: Coenagrionidae): Morphometric and genitalic differentiation. Annals of the Entomological Society of America 85: 662-670.

Luebke, H.J., Scriber, M.J. and Yandell, B.S. 1988. Use of multivariate discriminant analysis of male wing morphometrics to delineate a hybrid zone for *Papilio glaucus glaucus* and *P.g. canadensis* in Wisconsin. American Midland Naturalist 119: 366-379.

McDade, L. 1990. Hybrids and phylogenetic systematics I. Patterns of character expression in hybrids and their implications for cladistic analysis. Evolution 44: 1685-1700.

Muoneke, M.I. and Maughan, O.E. 1991. Multivariate morphometric analysis of striped bass, white bass and striped bass x white bass hybrids. North American Journal of Fisheries Management 11: 330-338.

Neff, N.A. and Smith, G.R. 1979. Multivariate analysis of hybrid fishes. Systematic Zoology 28: 176-196.

Rieseberg, L.H. and Ellstrand, N.C. 1993. What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Sciences 12: 213-241.

Spence, J.R. 1986. Relative impaacts of mortality factors in field populations of the waterstrider *Gerris buenoi* Kirkaldy (Heteroptera: Gerridae). Oecologia 70: 68-76.

Spence, J.R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Canadian Journal of Zoology 68: 1770-1872.

Sperling, F.A.H. and Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370-1383.

Sperling, F.A.H., Spence, J.R. and Andersen, N.M. 1997. Mitochondrial DNA, allozymes, morphology, and hybrid compatability in *Limnoporus* water striders (Heteroptera: Gerridae): Do they all track species phylogenies? Annals of the Entomological Society of America 90: 401-415.

Strauss, R.E. 1986. Natural hybrids of the freshwater sculpins *Cottus bairdi* and *Cottus cognatus* (Pisces: Cottidae): Electrophoretic and morphometric evidence. American Midland Naturalist 115: 87-105.

Szymura, J.M. and Barton, N.H. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45: 237-261.

Warwick, S.I., Thompson, B.K. and Black, L.D. 1992. Hybridization of *Carduus nutans* and *Carduus acanthoides* (Compositae): morphological variation in F1 hybrids and backcrosses. Canadian Journal of Botany 70: 2303-2309.

ECOLOGICAL CHARACTERIZATION AND DISTRIBUTION OF HABITATS IN A WATER STRIDER HYBRID ZONE

INTRODUCTION

Habitat characteristics and habitat availability may influence the width and position of a hybrid zone. Habitat characteristics can influence the geographic distribution of parental species and hybrids if selection for divergent traits occurs along a broad environmental cline (Endler 1977) or within different habitat types scattered across a heterogenous landscape (mosaic model: Harrison 1986). Genetic structure in hybrid zones may also be influenced by habitat availability, if dispersal between populations is restricted due to a lack of habitats or low population density (tension zone model: Barton and Hewitt 1985). Although both genetic incompatibilities and environmental factors may considerably influence hybrid zone structure, their relative contributions are not clearly understood for most hybrid zones. In particular, there is little empirical data to assess the influence of habitat availability on hybrid zone structure.

Habitat distribution within a hybrid zone may create a patchy population structure if each species is associated with a different habitat type (Harrison 1986). Associations between genotype and habitat characteristics have been reported for many hybridizing species [e.g. in northern flickers (Moore and Price 1993), grasshoppers (Shaw et al. 1993), field crickets (Rand and Harrison 1989), big sagebrush (Wang et al. 1998) and fire-bellied toads (MacCallum et al. 1998)]. A variable distribution of preferred habitat types for hybridizing species may influence regional patterns of gene exchange. For example, under this hypothesis, in regions where there are habitats preferred by both

species, the extent of hybridization will be relatively low when compared to another region where only one habitat type is found (MacCallum et al. 1998).

Habitat distribution can also influence patterns of hybridization through its effect on population density (Nichols and Hewitt 1986). The tension zone model of hybrid zone structure describes clines maintained by a balance between dispersal and selection against hybrids (Barton and Hewitt 1985). Thus, tension zones are expected to lie in areas of low population density, where there is a balanced influx of genes from either side (Hewitt 1975). At the extreme of low habitat density, a lack of suitable habitats can act as a physical barrier to dispersal and gene flow between populations. A less extreme case of low population density can limit introgression because few individual organisms are available for hybrid interactions. For example, locally low population density may limit introgression between fragmented populations of two katydid species in the Potomac hybrid zone (Shapiro 1998) and may limit the spread of advantageous alleles in a hybrid zone between herbaceous perennials in the genus *Piriqueta* (Martin and Cruzan 1999). Conversely, high population density tends to occur when habitats are relatively close together, resulting in increased opportunities for hybridizing.

Both habitat characteristics and habitat distribution have been hypothesized to influence the structure of a hybrid zone in western Canada between two water strider species, *Limnoporus notabilis* and *L. dissortis* (Spence 1990, Sperling and Spence 1991, Klingenberg et al. 2001). The width of this hybrid zone is up to 600 km wide (Sperling and Spence 1991), much wider than expected based on coarse estimates of dispersal capacity (Fairbairn and Butler 1990, Spence 2000). The extent of gene exchange is

regionally variable (Sperling and Spence 1991); in the interior of British Columbia the parental species appear to coexist without merging fully, while along the east slopes of the Rockies in western Alberta there is a steep cline in body length and alleles at four allozyme loci. Ecological factors may explain the presence of an "island" of hybridized *dissortis*-like populations (Spence 1990, Sperling and Spence 1991) in central British Columbia, where the arid environment is similar to areas where *L. dissortis* are found further to the east. Likewise, environmental features may direct the movement of this hybrid zone as suggested by analysis of samples collected a decade apart (Klingenberg et al. 2000). Although habitat characteristics and habitat abundance have been suggested to explain patterns of hybridization within this hybrid zone, they have not been well characterized.

In this study I assess the hypothesis that species-specific habitat preferences and habitat availability help account for the distribution of parental species and their hybrids across the east slopes of the hybrid zone. This hybrid zone is well suited to addressing questions about the influence of habitat availability because *Limnoporus* occupy discrete habitat patches. On a broad scale, I characterize habitats by vegetation and physical parameters and ask if *L. dissortis*, *L. notabilis* and their hybrids are found in different habitat types. I also quantify regional variation in habitat availability across the hybrid zone. At a broad scale, species composition within habitats is described using discriminant functions developed from morphometric traits (see Chapter 3). At a smaller scale, I assess habitat availability along a transect into the hybrid zone and use ecological traits to distinguish between habitats associated with different abundances of

Limnoporus. The results of our habitat sampling at a smaller scale are interpreted in light of previous work (see Chapter 2) which describes a region of transition for diagnostic alleles along a sampling transect.

METHODS

Sampling transects and collections

Limnoporus habitats were sampled along three transects oriented in a north-south direction within Alberta and western British Columbia (Figure 4-1). This sampling strategy was intended to encompass habitats containing hybridized populations in the foothills, populations that were entirely *L. notabilis* along the west slopes of the Rockies, and *L. dissortis* populations in eastern Alberta. I also sampled habitats along an east-west transect across the hybrid zone. Transects were defined by existing roads, and habitats were spotted while driving. I stopped at all locations with potentially suitable *Limnoporus* habitat; i.e. any pond or slow-moving water body with some surface or shoreline vegetation (Spence 1981). Each location was inspected for presence of *Limnoporus* and distances between inspected sites were recorded to the nearest kilometre. When habitats were less than one kilometre apart, the separation distance was rounded to the nearest full integer, 0 or 1. In habitats where *Limnoporus* were abundant, specimens were collected using hand nets, kept alive on moist paper towels, and transported back to the laboratory for morphometric and molecular work. In habitats where *Limnoporus* were present but not abundant, I recorded the presence of *Limnoporus* but did not collect them.

Species specific habitat preferences?

Assessing hybridization within habitats

I used morphometrics to assess the extent of hybridization within habitats where *Limnoporus* were abundant. Discriminant functions (see Chapter 3) were used to classify individuals as *L. dissortis*, *L. notabilis* or hybrid, based on 3 morphometric characters. Only males were used to assess hybridization morphometrically because males of the parental species and their hybrids are slightly better distinguished than are females (see Chapter 3). Gerrid populations in each habitat could then be characterized by the percent composition of *L. notabilis*, *L. dissortis* and hybrid males.

Water striders collected in June and August 1999, were measured. Two generations are represented in these samples; June collections represent overwintered, post-diapause adults, and August collections represent a summer generation of bugs born the same year and destined for winter diapause (Spence and Scudder 1980, Spence 2000).

Habitat Descriptions

I characterized habitats for populations where I had also collected specimens (i.e. only habitats with abundant *Limnoporus*). Vegetation composition, water properties and permanence were assessed at each site as traits important for *Limnoporus*. Vegetation structure influences maneuverability on the water surface (Spence 1981) and provides a substrate for water striders to lay eggs on (Spence 1986). Conductivity and pH may influence vegetation growth, composition of the aquatic community and hatchability of gerrid eggs, which are usually immersed throughout development (Spence and Scudder

1980). Habitat permanence is important because *Limnoporus* colonize temporary ponds early in the season, perhaps reducing interspecific competition for food (Spence and Scudder 1980, Spence 2000), avoiding egg parasitism (Spence 1986) and building up nutritional reserves (Spence 2000). Later in the summer, they disperse to more permanent habitats that are suitable for breeding (Spence 2000).

Vegetation composition was described to species, when possible, and was measured as the percent coverage of an area within a 50 cm^2 quadrat. Some plants, including grasses, sedges and rushes, could not be reliably identified to species and were grouped by family. Ten quadrat replicates were sampled in locations haphazardly chosen along the perimeter of each habitat in the zone of highest gerrid activity (Spence 1980). Thus, vegetation composition was described for a subset of the total pond area, in areas where there was aquatic vegetation. Plot cover per species was described as the average of the 10 quadrats. In the laboratory, pH and conductivity of water samples were measured with an Accumet AR25 meter and a CDM 83 conductivity meter, Radiometer, Copenhagen. Conductivity estimates were standardized to 25°C (Gardiner and Dackombe 1983).

Total habitat area was coarsely estimated for small ponds by pacing out the habitat boundaries and using stride length to estimate linear dimensions. This was not possible for streams and large water bodies where only a small portion of the total habitat area was accessible. These area estimates were translated into size categories (1= small or $<100m^2$, 2= medium or between $100m^2$ and $500m^2$, 3= large or $>500m^2$) to coarsely distinguish among habitats where *Limnoporus* were collected.

Loss of habitat due to drying was estimated by measuring the recession of the water's edge from early to late summer. Four woodens stakes were placed haphazardly around the perimeter of the habitat in June, and in August the distance from each stake to the water's edge was recorded. I also estimated changes in water depth within the area where I had collected *Limnoporus*. In early and late summer I indicated water levels on a wooden stake, placed within the *Limnoporus* habitat, with a notch filed into the wood. I measured the distance between notches as the change in depth. The absolute value of the average of habitat loss measurements divided by the change in depth provided an index of habitat permanence (habitat loss (cm) per change in depth (cm)); negative values were assigned to ponds with an overall loss in habitat and depth and positive values were assigned to ponds that increased in area and depth.

Habitat availability across the hybrid zone

I estimated the mean distance separating habitats where *Limnoporus* were discovered. Mean distances between habitats were calculated for each of the north-south transects using recorded kilometre readings for inspected sites.

I compared the regions of these transects with and without hybrid *Limnoporus* to determine if patterns of hybridization are associated with habitat availability. Specifically, because regions with a lack of suitable habitats have low population densities that may trap hybridized populations, I was interested in whether the mean distance separating *Limnoporus* sites was greater in regions with hybridization.

Habitat availability and introgression patterns

I assessed habitat availability along Highway 16, from Edson to Jasper National Park in June 2000, to determine if lack of suitable habitats can account for a transition from *dissortis*-like to *notabilis*-like alleles (Chapter 2) that occurs west of Hinton toward Jasper National Park. As with the other transects , I stopped at all sites suitable for *Limnoporus*. For this work, I described all inspected habitats, whether or not *Limnoporus* were actually discovered. Presence of numerous unoccupied habitats that are apparently suitable for *Limnoporus* would rule out lack of habitats as a significant barrier to gene flow in the hybrid zone. As before, I recorded distances between inspected sites to the nearest kilometre. To estimate relative *Limnoporus* abundance within sites, I recorded the time spent collecting and the number of individuals caught (Spence 1980).

For this work, structural characteristics of vegetation rather than species composition were used to categorize habitats because analysis of data from 1999 did not show a strong response by *Limnoporus* species to vegetation composition described by species or pooled species groups. Habitat structure is an important factor influencing gerrid species distributions (Spence 1981, 1983), and might better account for *Limnoporus* species distributions. At every site I estimated 1) area, 2) total percent cover of water surface by vegetation, 3) percent of water surface covered by small floating plants (e.g. duckweed, white water buttercup), 4) percent of water surface covered by large floating plants (e.g. lily species), 5) percent of water surface covered with emergent vegetation, 6) percent of water surface covered with submerged vegetation (reaching the water surface), 7) percent of shoreline covered with vegetation, 8) maximum depth and 9) spacing of plants. All characteristics were visually estimated except area and depth. Area

was measured as previously described. Maximum depth was evalulated along a scale of 0-2: 0)"very shallow" (less than knee deep, about 0.5 m), 1)"shallow" (less than about 1.3 metres deep or chest level), and 2) greater than chest depth.

In categories 5 and 7, I further differentiated 3 classes of plant height within the emergent and shoreline vegetation: 1) percent of emergent plants < 15 cm, 2) percent of emergent plants 15 to 100 cm, 3) percent of plants >100 cm. Spacing of vegetation was described with 4 categories: 0) mostly open water with some sparsely distributed surface vegetation, 1) moderately spaced surface vegetation (stems approximately greater than 10 cm apart), 2) closely spaced surface vegetation (stems approximately less than 10 cm apart), 3) clumped distribution of surface vegetation, and 4) vegetation only along the shoreline.

For habitats with emergent vegetation, I also measured the number of emergent stems and stem width, averaged over three replicates. To measure the number of emergent stems, a location within the emergent vegetation was haphazardly chosen to count the number of stems along 100 cm of a ruler. Stem width, averaged over three replicates, was measured with a ruler at the water surface of haphazardly chosen vegetation stems.

Multivariate ordination

Habitat descriptions and species composition

Principal components analysis (PCA) was used to describe the variation observed for environmental variables. Conductivity values were log-transformed prior to analysis.

Permanence was coded on a scale of 0-3; sites that had dried up completely by August were given a score of 0, sites that had decreased in area and depth were coded as 1, sites that stayed the same were given a 2 and sites that increased in area and depth were coded as 3. Vegetation cover was transformed on an octave scale (Gauch 1982) which is essentially logarithmic to the base 2, with 10 values between 0-9: 0 (0%), 1 (0-0.5%), 2 (0.5-1%), 3 (1-2%), 4 (2-4%), 5 (4-8%), 6 (8-16%), 7 (16-32%), 8 (32-64%), 9 (64-100%). This scale provides a level of accuracy appropriate for visual estimates of species abundances and puts dominant species on a more equitable footing with rarer species. Although I did not classify all vegetation to species, transforming the cover data to this scale effectively summarizes coarse visual estimates. Sites on PCA scatterplots were coded according to species composition.

Habitat characteristics and Limnoporus abundance

PCA was used to analyse differences among habitats with *Limnoporus* and habitats without *Limnoporus* in terms of ecological variables. Percent cover data was transformed on the octave scale (Gauch 1982) and area was coded by size classes described above. Abundance was based on capture rate in June, when vegetation was assessed.

RESULTS

***Limnoporus* populations and habitats**

Limnoporus were collected from 40 populations (Figure 4-1 and Table 4-1). Few *Limnoporus* populations were found in south-eastern British Columbia and across most of the transect covering eastern Alberta.

I did not find any populations that were entirely *L. notabilis*, although I did find populations that were entirely *L. dissortis* (Table 4-1, Figure 4-2). Two main regions of hybridization were detected. One region included populations located at the northern end of the transect along the west slope of the Rocky Mountains. These populations were mostly *L. notabilis*, with some hybrids and a very low percentage of *L. dissortis* (Table 4-1). The second region of hybridization was found along the eastern foothills of the Rocky Mountains, in the southwest corner of Alberta. Here, populations contained both *L. dissortis* and *L. notabilis*, with varying proportions of hybrids (Table 4-1). These results appear to contrast with previous work that identified hybrids in the Hinton region (see Chapter 2) because the population in this study (site 30) that is closest to Hinton is characterized entirely by *L. dissortis* individuals (Figure 4-2). However, site 30 is about 45 km east of Hinton, in the same region as a population described as entirely *L. dissortis* using genetic markers (Chapter 2).

All 28 habitat variables across 39 sites sampled within and adjacent to the hybrid zone were considered in a PCA. One site (#10) was removed from the ordination analysis because data about permanence were missing. A few sites (n=3) showed an increase in area with a decrease in depth and so did not fit into the permanence categories (0-3), as defined in the Methods section. These sites were located in regions with heavy summer rainfall; it is possible that erosion of basin contours allowed water to spread over a larger

area while decreasing the overall depth. Alternatively, errors in my earlier assessment of either area or depth may account for the unusual results. I grouped these sites with permanence group 3, to reflect the overall increase in aquatic area within these sites. Initial analysis showed site #33 as an outlier positioned at the top of the PC2 axis, distinguished from the rest of the sites mainly by presence of white water buttercup. Removing the outlier in a second PCA did not achieve greater separation of the sites, so I used the first ordination results to interpret the data.

Each site was coded according to water strider species composition; I was not able to simply compare *L. dissortis* and *L. notabilis* habitats since no populations consisted of all *L. notabilis* individuals. Instead, I grouped habitats into three categories: 1) all *L. dissortis*, 2) greater than 85% *L. notabilis* or 3) a mixed population containing *L. dissortis* and/or *L. notabilis* and/or hybrids. Comparisons between *L. dissortis* and *L. notabilis* habitats are limited by the small sample size (n=2) of habitats with >85% *L. notabilis* present, although they are somewhat separated along the PC1 axis (Figure 4-3). Habitats with "pure" *L. dissortis* are not distinguished from habitats containing hybrids in a PCA scatterplot (Figure 4-3).

Even with variables in three main classes, habitat variation was complex. The first 10 principal components accounted for 74.3% of the variability in habitat conditions (Table 4-2). The first principal component explains 15% of the variability between habitats and largely represents a gradient of conductivity and pH, with lower values of both variables on the left side of the axis. PC2, accounting for an additional 10.7% of the variability, mostly described differences in vegetation between sites. High PC2 scores

indicated sites with aquatic vegetation such as white water buttercup, duckweed, floating grass, and *Potamogeton*. These results suggest that association of *Limnoporus* species with somewhat different but overlapping habitats is not an important factor in explaining the *Limnoporus* hybrid zone.

Habitat availability across the hybrid zone

Overall, the probability of finding a habitat containing *Limnoporus* differed significantly among regions ($X^2 = 9.23$, $p = .01$). *Limnoporus* were rarest in eastern Alberta, where they were found in only 21 out of 74 (proportion = 0.28) inspected habitats. Most of the area without *Limnoporus* in eastern Alberta is located in dry grassland, while the area with *Limnoporus*, at the northern edge of the eastern Alberta transect, was found in aspen parkland. The proportion of inspected habitats with *Limnoporus* was similar between the transect along the western front of the Rockies (35 out of 72 sites, proportion = 0.49) and along the eastern foothills of the Rockies (39 out of 77 sites, proportion = 0.51). Still, *Limnoporus* were found in only about half of the habitats that appeared to be suitable.

The three north-south transects were not significantly different from each other in the mean distance separating habitats with *Limnoporus* (ANOVA, $p = 0.36$). The mean distance separating habitats with *Limnoporus* present was greatest along the west slopes (mean +/- S.E. = 43.5 +/- 10.0 km). Mean distances were similar in the foothills of the Rockies and in eastern Alberta, with an average of 30.2+/- 5.3 km and 30.4 +/- 6.3 km between *Limnoporus* sites, respectively.

Habitat availability v.s. species distributions

Morphometric analysis revealed that two transects, along the west side of the Rockies and along the eastern foothills, were distinguished by regional differences in the extent of hybridization. Along the western front of the Rockies, hybridized populations were discovered at the northern end of the transect, although one population was comprised of mostly *L. dissortis*. However, populations comprised of *L. notabilis* were found further to the south. In contrast, along the eastern foothills of the Rockies, the southern end of the transect contained varying proportions of hybrids, while localities in the northern part of the transect were almost entirely comprised of *L. dissortis*.

These observations prompted the following test. I compared habitat availability within the hybridized and non-hybridized regions of the west and east slopes to see if hybridized regions are located in areas of low population density. Sampling localities in the western transect where *Limnoporus* hybrids were found, were separated by a lower mean distance (mean +/- SE= 20.6 +/- 6.1 km) and a smaller range of distances (0-59 km) compared to the non-hybridized region of this transect (mean= 79.3 +/- 27.4 km, range: 6-221 km) (t-test, p=0.04). However, along the eastern foothills transect, *Limnoporus* habitats in the hybridized (mean +/- 1SE: 34.5 km +/- 7.9 km, range: 3-113 km) and non-hybridized (mean: 27.4+/- 7.8, range: 1-120 km) portion of the transect did not differ statistically from each other with respect to distance separating the sites (t-test, p=0.53).

Habitat availability between Jasper National Park and Edson

Habitat descriptions for sites with and without Limnoporus

Forty-two sites sampled between Edson and Jasper National Park and seventeen habitat variables were included in a PCA (Figure 4-4). A sharp transition from *L. dissortis* to *L. notabilis* markers (mtDNA and one nuclear marker) occurs within this transect (Chapter 2). Assessing habitat availability here could determine if the sharp trasition is acccounted for by a lack of suitable habitats. Each habitat was characterized by *Limnoporus* abundance estimates grouped into three categories: absent (0 caught), rare (<1 caught per minute) and abundant (>/= 1 caught per minute).

Ten principal components explained 91.7% of the total variance between sites. In general, sites with *Limnoporus* were separated from sites without *Limnoporus* along PC1. PC1 explained 22.5% of the variability and represents a gradient from *Limnoporus* habitats that are relatively deep with shoreline vegetation between 15 to 100 cm high, to habitats without *Limnoporus* that are characterized with shoreline and emergent vegetation less than 15 cm high (Table 4-3). PC2 explained 15.1% of the variability, and somewhat distinguishes habitats where *Limnoporus* were abundant. All sites where *Limnoporus* were abundant clustered at the lower half of PC2; lower values of PC2 are associated with emergent vegetation between 15 and 100 cm high and a high percentage of the water surface covered with vegetation. All high abundance habitats had the highest class of percent coverage for emergent vegetation that was greater than 15 cm but less than 100 cm tall.

These results distinguish lentic, vegetated habitats that are suitable for *Limnoporus* from those that are not suitable (Table 4-4). Interestingly, most of the

localities with abundant *Limnoporus* cluster together (Figure 4-4). Only 2 localities clearly cluster with this group (i.e. they are suitable habitats) but were unoccupied by *Limnoporus*; one locality was found between Edson and Hinton, and the other was located between Hinton and Jasper.

Habitat availability

I examined habitat availability between Edson and Hinton and between Hinton and Jasper National Park. If habitat availability can explain the intially sharp transition from *L. dissortis* to *L. notabilis* alleles, I would expect fewer suitable localities between Hinton and Jasper compared to habitat availability between Edson and Hinton. Alternatively, a similar numbers of suitable habitats between the two sections of the transect, with a greater proportion of suitable but unoccupied habitats between Hinton and Jasper National Park, would point to intrinsic genetic incompatabilities as the more likely explanation for the transition pattern.

Between Edson and Hinton (including a population in Hinton) I found 14 habitats with *Limnoporus*, separated by a mean (+/- SE) distance of 5.4 +/- 2.2 km. In comparison, between Hinton and the eastern side of Jasper National Park, I discovered 4 habitats with *Limnoporus*, separated by a mean (+/- SE) distance of 10.0 +/- 4.5 km. Although this trend is in the right direction to suggest spatial effects, differences between means are non-significant (t-test, p=.35). However, all 4 habitats west of Hinton also had a low abundance (<1 caught/minute) of *Limnoporus;* within two of these sites I found

only 1 and 3 individuals after 10 minutes of searching. In contrast, between Edson and Hinton, *Limnoporus* were abundant (>1 caught/minute) in 7/13 habitats.

DISCUSSION

Barriers to gene flow across a hybrid zone result when there is: a) selection against hybrids that removes genes that have introgressed from adjacent populations (selection acts equally on all genes); b) selection against some alleles in a foreign genetic background; c) selection against genotypes in the "wrong" environment; d) a physical barrier (e.g. an impassable landscape feature or a lack of suitable habitats). I assessed the possible contributions of the latter two mechanisms within a *Limnoporus* hybrid zone in western Canada. The results suggest that habitat effects on *L. notabilis*, *L. dissortis* and their hybrids, are not important factors in this hybrid zone because species distributions were not associated with particular habitat characteristics. However, the distribution and quality of habitats within the hybrid zone may be significant. Combined with selection against some genotypic combinations (Spence 1990, Sperling and Spence 1991, see Chapter 2), an apparent lack of good habitats along the east slopes may account for the "patchy" spatial structure of this hybrid zone.

Habitat use

The conclusion that there is no difference in habitat use between species must be tempered because I did not sample a region that included only *L. notabilis* populations. This omission was not by design but the result of more introgression in western regions than predicted at the outset of the study. On the western edge of the hybrid zone,

Limnoporus habitats that do not contain introgressed *dissortis*-like alleles may be relatively rare, compared to habitats hosting pure *L. dissortis* along the eastern edge of the zone. Asymmetrical introgression, from *L. dissortis* populations into those of *L. notabilis*, has been observed using data from body lengths (Spence 1990) and four allozyme loci (Sperling and Spence 1991). In addition, a recent study using sequence data shows that at least one *dissortis*-type nuclear marker is passing into *L. notabilis* populations more freely than previously thought (see Chapter 2). Therefore, it is possible that "pure" *L. notabilis* populations are restricted to the western edge of the species population range in Canada.

Our study leaves open the possibility that environment-dependent selection acts in a similar way for *L. dissortis* and hybrids but differently for *L. notabilis*. However, it seems unlikely that genotype-environment interactions significantly influence the distribution of hybrids and *Limnoporus* parentals. First, hybrids are found in habitats that are similar to those where only *L. dissortis* are found. Second, patterns of hybridization do not follow the contours of the Rocky Mountains but continue through the transition area into British Columbia. One possibility is that *Limnoporus* water striders respond to environmental features that were not measured, including climatic gradients of precipitation and temperature.

Does low habitat availability influence patterns of introgression?

Results of this study show that habitat availability, and concomitantly, population density, varies by region across the hybrid zone. At a broad scale, habitat availability may partially account for regional patterns of morphometric variation. Within

the northern portion of the western slopes transect where populations appear to hybridize, suitable habitats are relatively close together. Patchy population density in a contact area can broaden hybrid zones, depending on the scale of patchiness (Butlin et al. 1991). Patchy populations, combined with extinction and colonization, may effectively broaden the hybrid zone along our transect west of Jasper National Park. Klingenberg et al. (2000) provided evidence of a broadening area of hybidization in central British Columbia, over the course of about a decade. However, it is not clear if the expansion was associated with changes in habitat availability. Unfortunately, I did not estimate habitat availability further west into the interior of British Columbia where Sperling and Spence (1991) found an isolated region with a high frequency of *L. dissortis*.

In contrast, patchy populations may exist at a larger scale in the southern portion of the transect along the west slopes of the Rockies, where I found that suitable habitats were widely separated. In this region, I found no abundant *Limnoporus* populations. This region may create a sharp gradient in population density across the mountains, and concentrate hybridization within the eastern foothills along the southern portion of the foothills transect. Regions with a lack of suitable habitats represent extreme cases of density variation (Nichols and Hewitt 1986), where tension zones are expected to become trapped (Barton and Hewitt 1985). For *Limnoporus*, the landscape in eastern Alberta or southeastern British Columbia may act as physical barriers that effectively block gene flow between habitat patches on either side of the barrier.

Both theoretical (Nichols 1989) and empirical (e.g. subspecies of *Podisma pedestris:* Nichols and Hewitt 1986, subspecies of *Chorthippus parallelus*: Hewitt 1989)

evidence show that local changes in population density may account for regional differences in cline width. Patchy population density can broaden hybrid zones, especially when the effective population size is smaller and the density gradient is shallow (Nichols and Hewitt 1986, Nichols 1989). However, the scale of patchiness is an important consideration because large habitat patches can create steep density gradients, and concentrate clines at a barrier to dispersal (Nichols 1989, Butlin et al. 1991). At the lower extreme of density variation, gene flow may be completely blocked (Barton 1979, Nichols and Hewitt 1986).

Knowledge of *Limnoporus* dispersal capacity is central to placing estimates of habitat spacing within a meaningful biological context (i.e. what distance constitutes a barrier to dispersal?). Unfortunately, there are no direct estimates of dispersal capacity for *Limnoporus* species, but movement between habitats is frequent (Spence 2000) and occurs over at least several kilometres (Fairbairn and Butler 1990, Spence 2000).

Other water strider species (Preziosi and Fairbairn 1992) and insects (katydids: Shapiro 1998, whirlygig beetles: Nürnberger and Harrison 1995, field crickets: Harrison 1986, fire ants: Shoemaker 1998) show dispersal patterns that are limited by a patchy distribution of suitable and ephemeral habitats. The genotypes of individuals first colonizing such a habitat may be more important in determining the genetic character within habitats (Shoemaker 1998) than is overall low hybrid fitness due to genetic effects (tension zone: Barton and Hewitt 1985) or the effects of different habitat types (mosaic model: Rand and Harrison 1989). Given the relatively low proportion of locally suitable habitats occupied by *Limnoporus* populations, chance colonization could play a

significant role in promoting hybridization, even in regions where they are locally abundant.

Variation in habitat availability cannot explain all the patterns I observed. Along both the eastern foothills and the transect bisecting the hybrid zone, the mean distance separating *Limnoporus* habitats was not different in the hybridized or non-hybridized regions. However, factors other than the number and spacing of available habitats can also influence population density across a hybrid zone.

Does habitat quality influence introgression?

Our results show that, at a smaller scale, ecologically marginal habitats and low *Limnoporus* abundance coincide with a region where there is a transition between *L. dissortis* nuclear and mitochondrial markers to those of *L. notabilis* (see Chapter 2) along Highway 16. The mean distance separating habitats is greater, but not significantly so in a modest sample, within the transition area compared to an adjacent region. However, there is an apparent difference in the quality of habitats. West of Hinton, suitable habitats (n=4) were ecologically marginal and were occupied by *Limnoporus* at a low density. In this area we sampled only one suitable, unoccupied habitat. In ecologically marginal habitats, genotypes may mix over greater distances (e.g. *Podisma pedestris*: Nichols and Hewitt 1986). This may contribute to the breadth of the *Limnoporus* zone west of Jasper National Park and can be better assessed by expanding the sampling area.

Distinguishing the effects of physical or intrinsic barriers to gene flow is not clear-cut. Reduced population density and restricted gene flow can also arise if there is a

"hybrid sink" (Barton 1980). Gene flow is restricted in a hybrid sink when low hybrid fitness effectively removes genes that have introgressed from adjacent populations, resulting in low population density. Distinguishing between low population density that is caused by environmental factors or genetic incompatabilities depends on knowledge of the strength of selection against hybrids, the influence of habitat characteristics on population density, and the organism's dispersal capacity.

A hybrid sink hypothesis cannot be ruled out for the region of the hybrid zone I sampled west of Hinton. There is strong selection against hybrids, because 50% of hybrid progeny die (Spence 1990). However, while selection acts to eliminate most, if not all, female hybrids, surviving male hybrids are fertile (Spence 1990, Sperling and Spence 1991, Sperling et al. 1997). Genetic incompatabilities are not sufficient to overcome substantial introgression of *dissortis* alleles along the same transect through the hybrid zone I sampled for this study, at least at one nuclear locus (see Chapter 2). More convincing evidence for a hybrid sink would be a preponderance of habitats with a low density or absence of *Limnoporus* despite possessing characteristics of high-abundance habitats. Such evidence could be accumulated through detailed field surveys, now that the east slopes of the hybrid zone have been better defined.

Historical context

Variation in hybrid zone width may also be attributed to the time elapsed since secondary contact. In areas of recent contact, dispersal and colonization may have the greatest influence on hybrid zone structure, resulting in a relatively narrow zone width (Shoemaker et al. 1996). With prolonged contact, the area of hybridization may broaden

as factors such as competitive abilities, overall fitness of different genotypes (Shoemaker et al. 1996) or the time for neutral alleles to spread by diffusion (Shapiro 1998) increasingly influence genetic structure. *L. notabilis* and *L. dissortis* probably first diverged before the Pleistocene (Sperling and Spence 1990), when large tracts of Alberta and British Columbia were covered by glaciers (2 000 0000 - 10 000 yr B.P.), and came into contact sometime after the retreat of the last glacier. More recently, contact between species was probably influenced by anthroprogenic changes like road construction through mountain passes, or the appearance of agricultural ponds in the dry lands east of the mountains. These changes probably created *Limnoporus* habitats in regions where a lack of suitable habitats had previously kept the species apart. Thus, regional patterns of variation in the hybrid zone may reflect changes to the landscape that facilitated contact between species.

The occurence of many hybridizing taxa in a similar region of western Canada points to a shared history among taxa. The foothills of the Canadian Rockies are located within what has been described as one of the major suture zones of North America (Remington 1968), where a variety of organisms hybridize, including deer (Hornbeck and Mahoney 2000), foxes (Mecure et al. 1993), many bird species (Remington 1968), *Papilio* butterflies (Sperling 1987, 1990), *Cicindela* beetles (Spanton 1988), pine trees (Raup 1946), spruce trees (Raup 1946) and poplar trees (Rood et al. 1986). Diverse species pairs hybridizing in the same region suggest that formidable barriers to gene exhange kept floral and faunal communities from contacting each other until relatively recently (Remington 1968).

Population composition reflects patterns of dispersal

The modality of hybridized populations within the northern region of the west slopes is biased strongly towards *L.notabilis* individuals. The modality of populations along the transect through Jasper National Park suggests that most hybrids here result from backcrosses to female *L. notabilis*, with hybridization between "pure" species a rare event. This agrees with a scenario of male hybrids (see Chapter 2) dispersing out of the area where the species overlap, and backcrossing into *L. notabilis* populations further west of the Rockies. Asymmetrical introgression, from *L. dissortis* into *L. notabilis* populations, probably results from a combination of greater interspecific mating success for *L. dissortis* males and low survivorship of hybrid females.

Modality of populations in the southern foothills suggests that barriers to reproduction are greater, since both parental species are present with some intermediate hybrids. Although the possibility of reinforcement cannot be ruled out, it is more likely that the discriminant functions used to classify individuals morphometrically may have underestimated the proportion of hybrids within these populations. Backcrossed individuals cannot be reliably identified with discriminant functions, and are more likely than F_1 hybrids to be classified as one of the parental species (see Chapter 3). Interestingly, Sperling (1990) reported extensive hybridization between two species of *Papilio* butterflies in a similar region of the foothills in Alberta, with the frequency of hybrids declining gradually north of this region.

Future considerations

Regions with a low population density may experience more introgression between species than more densely populated regions in a hybrid zone (Nichols and Hewitt 1986). Thus, variation in population densities across the hybrid zone may result in a patchy spatial structure. Combining the views of two models of hybrid zone structure emphasizes the importance of tension zone processes (Barton and Hewitt 1985) that occur over a patchy spatial scale (Harrison 1986). An exciting aspect of studying population density within hybrid zones is assessing the effect of density-dependence on mate discrimination between species (Kaneshiro 1989). For organisms like *Limnoporus* that are frequently mixed and redistributed in response to habitats that are unpredictably formed and lost, differences in species density may commonly arise. For hybridizing species in localities with a small population size, this can be a powerful factor facilitating hybridization (Hubbs 1955, Avise and Saunders 1984). Our study presents a good starting point for addressing further questions about the influence of population density and population composition in the *Limnoporus* hybrid zone.

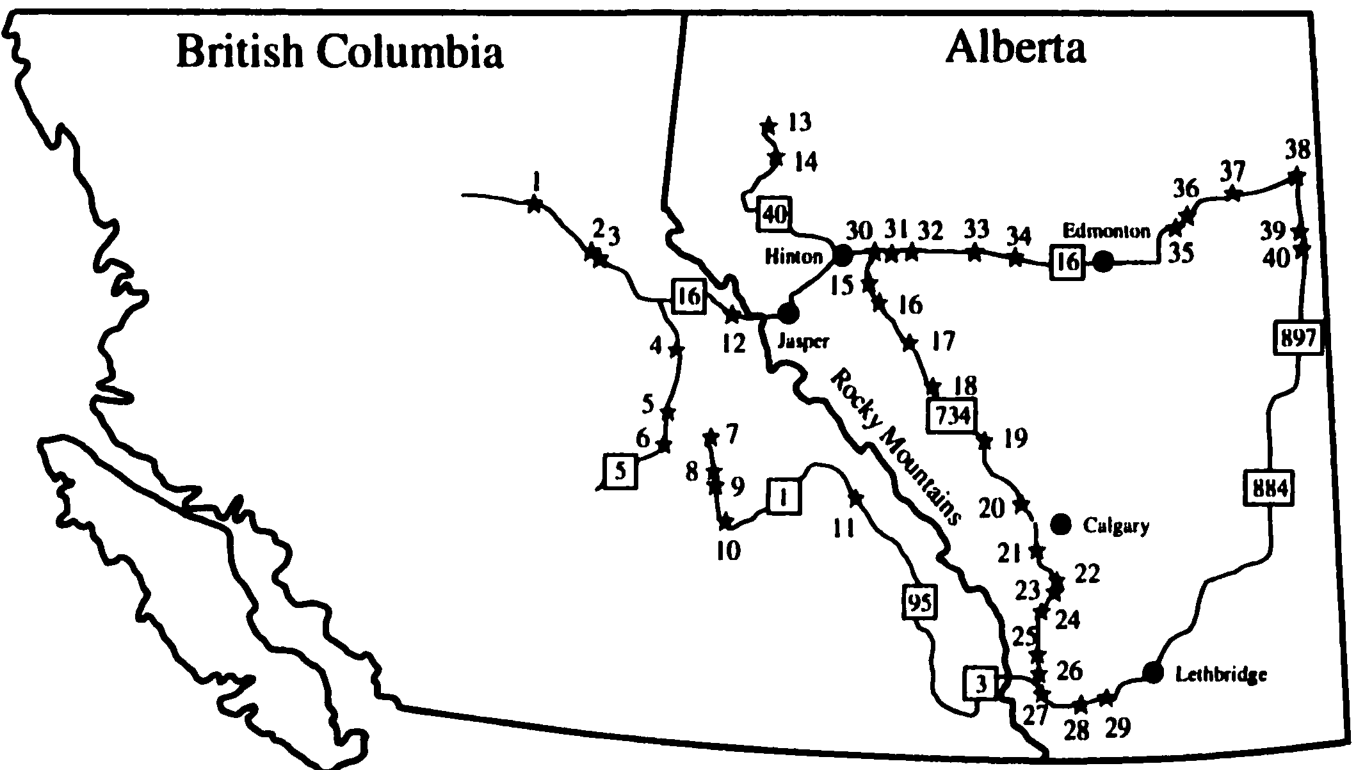

Figure 4-1: Map showing three north-south transects searched for *Limnoporus* habitats (transect 1: west side of the Rocky Mountains, from site 1 to highway 3, on the west side of the British Columbia-Alberta border; transect 2: eastern foothills of the Rockies, from site 13 to site 29; transect 3: eastern Alberta, from site 38 to Lethbridge). The east-west transect is shown from site 38 to site 12. Numbered stars indicate collection sites and towns are shown as circles for reference. Highway numbers are shown in boxes.

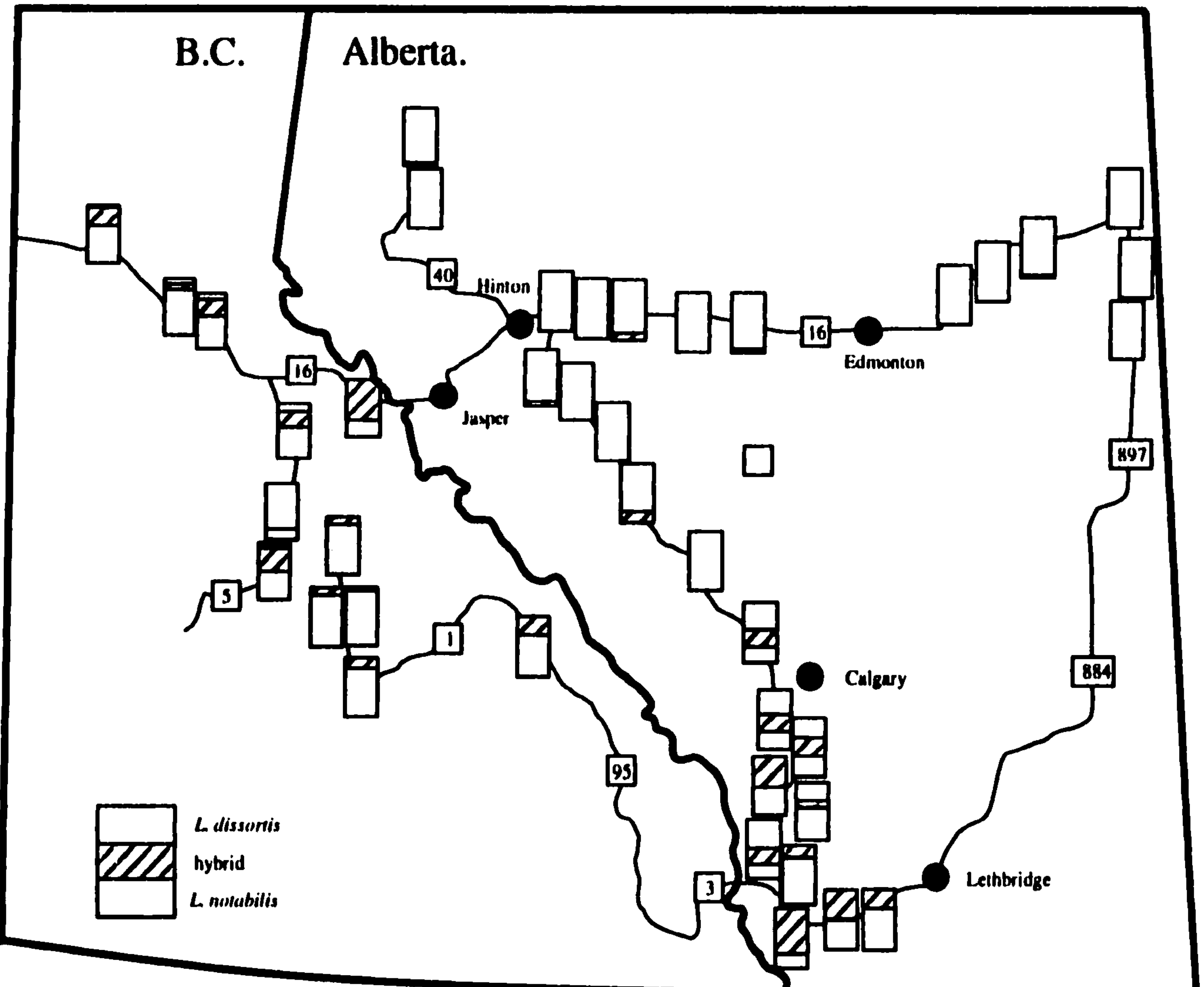

Figure 4-2: Map showing population composition, determined morphometrically, for sites where *Limnoporus* were collected. See histogram in lower left corner of map for interpretation. Highway numbers are shown in boxes.

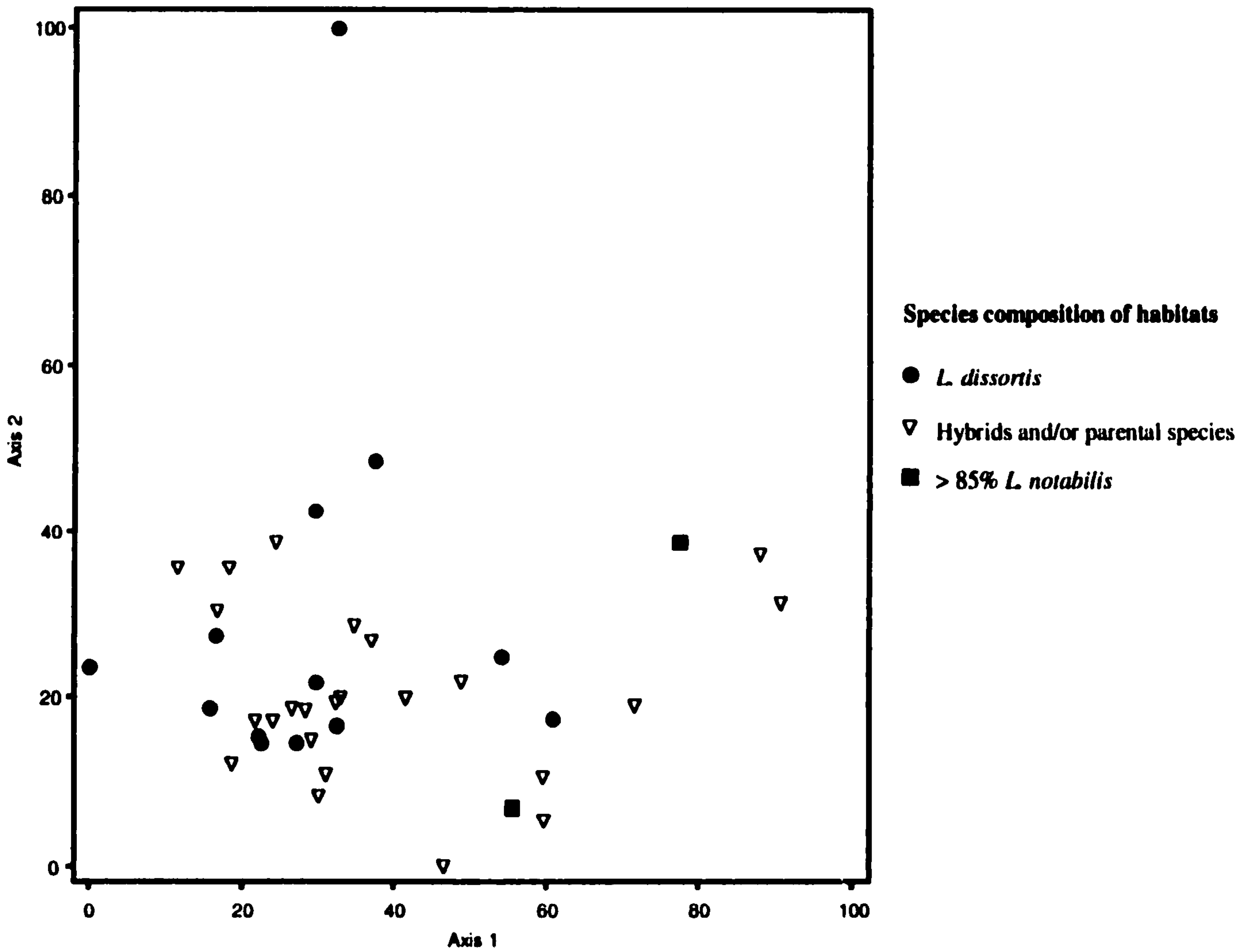

Figure 4- 3: Scatterplot of PC1 and PC2 values of 39 *Limnoporus* habitats in multivariate ecological space. Habitats are coded by species composition.

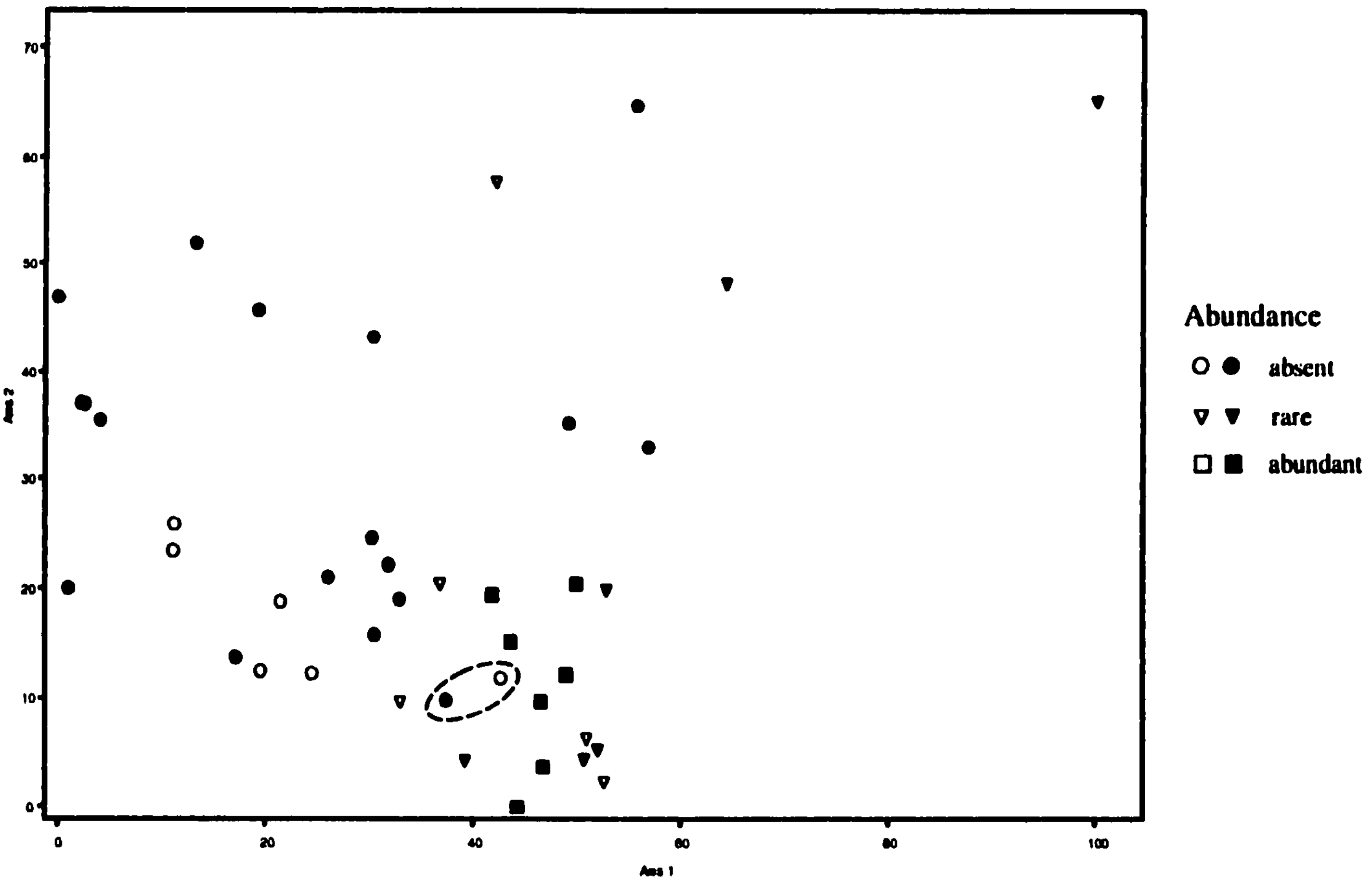

Figure 4-4: PCA scatterplot of 42 habitats inspected for *Limnoporus* between Edson and Jasper National park. Each habitat is coded by *Limnoporus* abundance and location relative to Hinton, Alberta. Open shapes indicate sites west of Hinton, and closed shapes represent sites east of Hinton. Two outlined shapes indicate habitats that were unoccupied by *Limnoporus* but cluster with suitable habitats. There were no abundant populations west of Hinton.

Table 4-1: Location of collecting sites, sample size and species composition as revealed morphometrically with discriminant functions. Locality number corresponds to Figure 4-1. LD=*L. dissortis*, LN= *L. notabilis*

				% species composition			
Locality	Location	Latitude	Longitude	LD	LN	hybrid	n
1	Hwy. 16, south of Prince George, B.C.	53.666°N	121.051°W	3	65.7	31.3	67
2	Hwy. 16, north of McBride, B.C.	53.396°N	120.469°W	10	80	10	30
3	Hwy. 16, north of McBride, B.C.	53.396°N	120.469°W	9.3	58.1	32.6	43
4	Hwy. 5, south of Valemount, B.C.	52.476°N	119.069°W	14.3	57.1	28.6	7
5	Hwy. 5, south of Valemount, B.C.	52.131°N	119.288°W	81.8	18.2	0	11
6	Hwy. 5,south of Blue River, B.C.	51.729°N	119.611°W	10	50	40	20
7	Hwy. 23, south of Mica Dam, B.C.	51.980°N	118.515°W	0	86.7	13.3	15
8	Hwy. 23, north of Revelstoke, B.C.	51.547°N	118.474°W	0	88.2	11.8	17
9	Hwy. 23, north of Revelstoke, B.C.	51.504°N	118.443°W	0	95	5	20
10	Hwy. 23, Revelstoke, B.C.	51.045°N	118.175°W	0	83.3	16.7	30
11	Hwy. 95, south of Golden, B.C.	51.179°N	116.767°W	0	70.6	29.4	17
12	Hwy 16, west of B.C./Alberta border	52.915°N	118.791°W	0	28.6	71.4	7
13	Hwy 40, south of Grande Prairie	54.876°N	118.740°W	94.4	0	5.6	18
14	Hwy 40, north of Grande Cache	54.601°N	118.699°W	100	0	0	45
15	Hwy 47, near Robb	53.291°N	116.947°W	91.7	0	8.3	24
16	Hwy 47, south of Robb	53.188°N	117.004°W	100	0	0	9
17	Hwy 734, Forestry Trunk Road	52.897°N	116.571°W	100	0	0	8
18	Hwy 734, Forestry Trunk Road	52.672°N	116.290°W	80	0	20	5
19	Hwy 734, Forestry Trunk Road	51.932°N	115.195°W	100	0	0	2
20	Hwy 40, near Cochrane	51.284°N	114.824°W	45.5	27.3	27.3	11
21	Hwy 762, south of Bragg Creek	50.844°N	114.508°W	43.8	31.3	31.3	16
22	Hwy 22, west of Black Diamond	50.710°N	114.301°W	33.3	33.3	33.3	6
23	Hwy 22, south of Black Diamond	50.601°N	114.230°W	33.3	55.6	11.1	9
24	Hwy 541, 30 km east of hwy 40 junction	50.546°N	114.370°W	0	50	50	4
25	Hwy 940, Forestry Trunk Road	50.058°N	114.425°W	50	25	25	4
26	Hwy 940, Forestry Trunk Road	49.784°N	114.481°W	0	80	20	5
27	Hwy 3, Crowsnest Pass area	49.634°N	114.534°W	0	25	75	4
28	Hwy 6, south of Pincher Creek	49.368°N	113.919°W	0	50	50	6
29	Hwy 505, southwest of Lethbridge	49.319°N	113.605°W	0	66.7	33.3	3
30	Hwy 16, 43 km west of Edson	53.535°N	117.039°W	100	0	0	31
31	Hwy 16, 29 km west of Edson	53.534°N	116.862°W	100	0	0	25
32	Hwy 16, 25 km west of Edson	53.541°N	116.809°W	88.9	0	11.1	9
33	Hwy 16, just west of Niton Junction	53.617°N	115.781°W	100	0	0	33
34	Hwy 16, 112 km west of Edmonton	53.608°N	115.175°W	92.3	0	7.7	26
35	Hwy 36, south of Two Hills	53.596°N	111.769°W	100	0	0	10
36	Hwy 36, south of Two Hills	53.614°N	111.770°W	100	0	0	18
37	Hwy 881, north of St. Paul	54.084°N	111.276°W	96.7	3.3	0	30
38	Hwy 659, east of Bonnyville	54.268°N	110.500°W	100	0	0	5
39	Hwy 897, eastern AB	53.959°N	110.405°W	100	0	0	6
40	Hwy 897, near site 39	53.952°N	110.404°W	100	0	0	25

Table 4-2: Results of PCA between *Limnoporus* habitat variables and derived principal components (PCs) for 39 habitats.

Habitat variables	Principal components	
	PC1	PC2
conductivity	-0.3985	0.0009
pH	-0.3159	-0.1880
permanence	0.2494	0.1456
area	0.1084	0.1496
arum-leaved arrowhead (*Sagittaria cuneata*)	0.0373	-0.1373
buck-bean (*Menyanthes trifoliata)*	0.2082	0.0424
marsh cinquefoil (*Potentilla palustris)*	0.2867	0.0877
alsike clover (*Trifolium hybridum)*	-0.1259	-0.0341
dead vegetation	0.2175	0.0650
common duckweed (*Lemna minor)*	-0.0990	0.3479
floating grass	-0.0288	0.3731
grass sp., Poaceae	-0.3090	0.0280
Horsetail/Mare's tail (*Equisetum/Hippuris vulgaris)*	0.0531	0.1320
unknown	-0.1214	0.0960
moss	0.1597	-0.1826
narrow-leaved bur-reed (*Sparganium angustifolium)*	-0.1900	-0.0080
open water	-0.1579	-0.0945
Potamogeton sp.	-0.1020	0.3883
rush sp., Juncaceae	0.0739	-0.2600
willow sp., Salix	0.2153	-0.1219
surface scum	-0.0280	0.2676
seaside arrow-grass, (*Triglochin maritima*)	0.0768	0.0342
sedge sp., Cyperaceae	0.3511	0.1318
small-fruited bulrush (*Scirpus microcarpus*)	0.1776	-0.1308
common cattail (*Typha latifolia*)	-0.0876	-0.0904
vernal water-starwort (*Callitriche verna*)	-0.0494	-0.0382
white-water-buttercup (*Ranunculus aquatilis var. capillaceus)*	-0.0174	0.4373
small yellow water-buttercup *(Ranunculus gmelinii)*	-0.1817	0.1265
% Variance Explained: Proportion	15.03	10.72
% Variance Explained: Cumulative	15.03	25.79

Table 4-3: Results of PCA analysis between structural habitat characteristics and derived principal components for 42 habitats sampled between Edson and Jasper National Park, Alberta.

Habitat variables	PC1	PC2
approximate area	0.2095	-0.2184
% of water surface covered with vegetation	0.0339	-0.3334
% water surface covered by small floating plants	0.2580	0.2341
% water surface covered by large floating plants	0.0368	-0.1363
% water surface covered by emergent plants	0.0539	-0.2107
% water surface covered by submerged plants	0.2728	0.2035
% shoreline covered by vegetation	0.1360	-0.0985
% shoreline plants 0-15 cm tall	-0.2992	0.0447
% shoreline plants15-100 cm tall	0.3337	-0.3444
% shoreline plants >100 cm tall	0.1939	0.2399
% emergent plants 0-15 cm tall	-0.3510	0.0513
% emergent plants15-100 cm tall	0.2635	-0.3791
% emergent plants>100 cm tall	0.2354	0.3865
maximum depth	0.3803	-0.0405
plant spacing	0.2060	-0.1123
average # stems of emergent vegetation per 100 cm	0.0001	-0.2555
average stem width of emergent vegetation	0.3430	0.3446
% Variance Explained: Proportion	22.5%	15.1%
% Variance explaine: Cumulative	22.5%	37.6%

Table 4-4: Range of pond conditions among sites were *Limnoporus* were found and where they were not found.

Variable	Ponds with Limnoporus (n=21) range			mean	Ponds without Limnoporus (n=28) range			mean
area	1 (<100m^2)	-	3 (>500m^2)	2.5	1 (<100m^2)	-	3 (>500m^2)	1.9
% H_2O surface covered by vegetation	3 (1-2%)	-	9 (64-100%)	7.2	0 (0%)	-	9 (64-100%)	6.0
% H20 surface covered by small floating plants	0 (0%)	-	3 (1-2%)	0.1	0 (0%)	-	5 (4-8%)	0.4
% H20 surface covered by large floating plants	0 (0%)	-	3 (1-2%)	0.1	0 (0%)	-	6 (8-16%)	0.4
% H20 surface covered with emergent vegetation	8 (32-64%)	-	9 (64-100%)	9.0	0 (0%)	-	9 (64-100%)	8.4
% H20 surface covered with submerged vegetation	0 (0%)	-	8 (32-64%)	1.2	0 (0%)	-	0 (0%)	0.0
% shoreline covered with vegetation	9 (64-100%)	-	9 (64-100%)	9.0	0 (0%)	-	9 (64-100%)	8.3
% shoreline vegetation <15cm. high	0 (0%)	-	9 (64-100%)	2.1	0 (0%)	-	9 (64-100%)	4.7
% shoreline vegetation 15 to 100 cm high	0 (0%)	-	9 (64-100%)	7.9	0 (0%)	-	9 (64-100%)	5.2
% shoreline vegetation >100 cm high	0 (0%)	-	8 (32-64%)	2.5	0 (0%)	-	9 (64-100%)	1.5
% emergent vegetation <15 cm. high	0 (0%)	-	9 (64-100%)	0.9	0 (0%)	-	9 (64-100%)	4.2
% emergent vegetation 15 to 100 cm high	5 (4-8%)	-	9 (64-100%)	8.6	0 (0%)	-	9 (64-100%)	5.6
% emergent vegetation >100 cm high	0 (0%)	-	9 (64-100%)	2.1	0 (0%)	-	9 (64-100%)	1.2
maximum depth	0 (< knee deep)	-	2 (>chest deep)	1.5	0 (< knee deep)	-	2 (>chest deep)	0.6
vegetation spacing	0 (mostly open water)	-	3 (clumped)	1.7	0 (mostly open water)	-	4 (shoreline veg. only)	1.1
average # stems of emergent vegetation/100cm	5.3	-	29.0	15.3	0	-	64.3	11.8
average stem width of emergent vegetation	2.2 mm	-	21.7 mm	5.9	0	-	15.7 mm	3.6
average # stems of large floating vegetation/100cm	0	-	0	0.0	0	-	19.7	0.7
average stem width of large floating vegetation	0	-	0	0.0	0	-	3.3	0.1
average # stems of small floating vegetation/100cm	0	-	0	0.0	0	-	54	1.9
average stem width of small floating vegetation	0	-	0	0.0	0	-	2.7	0.1

REFERENCES

Avise, J.C. and Saunders, N.C. 1984. Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers. Genetics 108: 237-255

Barton, N.H. 1979. Gene flow past a cline. Heredity. 43: 333-340.

Barton, N.H. 1980. The hybrid sink effect. Heredity 44:277-78.

Barton, N.H. and Hewitt, G.M. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113-48.

Butlin, R.K., Ritchie, M.G. and Hewitt, G.M. 1991. Comparisons among morphological characters and between localities in the *Chorthippus parallelus* hybrid zone (Orthoptera: Acrididae). Philosophical Transactions of the Royal Society of London, Series B: 334: 297-308.

Endler, J.A. 1977. Geographic Variation, Speciation, and Clines. Princeton University Press, Princeton, New Jersey.

Fairbairne, D.J. and Butler, T.C. 1990. Correlated traits for migration in the Gerridae (Hemiptera, Heteroptera): a field test. Ecological Entomology 15: 131-142.

Gardiner, R.V. and Dackombe, V. 1983. Geomorphological Field Manual. Geo. Allen & Unwin Publishers, London.

Gauch Jr., H.G. 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, New York, NY.

Harrison, R.G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56: 337-349.

Hewitt, G.M. 1975. A sex chromosome hybrid zone in the grasshopper Podisma pedestris (Orthoptera: Acrididae). Heredity 35: 375-387.

Hewitt, G.M. 1989. The Subdivision of Species by Hybrid zones. Pp. 85-110 *in* D. Otte and J.A. Endler, eds. Speciation and Its Consequences. Sinauer Associates Incorporated, Sunderland, Massachusetts.

Hornbeck, G.E. and Mahoney, J.M. 2000. Introgressive hybridization of mule deer and white-tailed deer in southwestern Alberta. Wildlife Society Bulletin 28: 1012-1015.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1-20.

Kaneshiro, K.Y. 1989. Natural hybridization in *Drosophila*, with special reference to species from Hawaii. Canadian Journal of Zoology 68: 1800-1805.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. Journal of Evolutionary Biology 13: 756-765.

MacCallum, C.J., Nürnberger, B., Barton, N.H. and Szymura, J.M. 1998. Habitat preference in the *Bombina* hybrid zone in Croatia. Evolution 52: 227-239.

Martin, L.J. and Cruzan, M. B. 1999. Patterns of hybridization in the *Piriqueta caroliniana* complex in central Florida: evidence for an expanding hybrid zone. Evolution 53: 1037-1049.

Mecure, A., Ralls, K., Koepfli, K.P., and Wayne, R.K. 1993? Genetic subdivisions among small canids: Mitochondrial DNA differentiation of swift, kit, and arctic foxes. Evolution 47: 1313-1328

Moore, W.S. and Price, J.T. 1993. Nature of Selection in the Northern Flicker Hybrid Zone and Its Implications for Speciation Theory. Pp. 196-225 *in* R.G. Harrison, ed. Hybrid zones and the Evolutionary Process. Oxford University Press, N.Y.

Nichols, R.A. 1989. The fragementation of tension zones in sparsely populated areas. American Naturalist 134: 969-977.

Nichols, R.A. and Hewitt, G.M. 1986. Population structure and the shape of a chromosomal cline between two races of a *Podisma pedestris* (Orthoptera: Acrididae). Biological Journal of the Linnean Society 29: 301-316.

Nichols, R.A. and Hewitt, G.M. 1988. Genetical and ecological differentiation across a hybrid zone. Ecological Entomology 13: 39-49.

Nurnberger, B. and Harrison, R.G. 1995. Spatial population structure in the whirlygig beetle *Dineutus assimilis*: evolutionary inferences based on mitochondrial DNA and field data. Evolution 49: 266-275.

Preziosi, R.F. and and Fairbairn, D.J. 1992. Genetic population structure and levels of gene flow in the stream dwelling waterstrider, *Aquarius (=Gerris) remigis* (Hemiptera: Gerridae). Evolution 46: 430-444.

Rand, D.M. and Harrison, R.G. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution 43: 432-439.

Raup, H.M. 1946. Phytogeographic studies in the Athabasca-Great Slave Lake region, II. J. Arnold. Arb., 27: 1-85.

Remington, C.H. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evol. Biol. 2: 321-428.

Rood, S.B., Campbell, J.S., Despins, T. 1986. Natural poplar hybrids from southern Alberta (Canada): I. Continuous variation for foliar characteristics. Can. J. Bot. 64: 1382-1388.

Shapiro, L.H. 1998. Hybridization and geographic variation in two meadow katydid contact zones. Evolution 52: 784-796.

Shaw, D.D., Marchant, A.D., Contreras, N., Arnold, M.L., Groeters, F., and Kohlmann, B.C. 1993. Genomic and environmental determinants of a narrow hybrid zone: cause or coincidence?. Pp. 165-195 *in* R.G. Harrison, ed. Hybrid zones and the Evolutionary Process. Oxford University Press, N.Y.

Shoemaker, D.D., Ross, K.G. and Arnold, M.L. 1996. Genetic structure and evolution of a fire ant hybrid zone. Evolution 50: 1958-1976.

Spanton, T.G. 1988. The *Cicindela sylvatica* group: Geographical variation and classification of the Nearctic taxa, and reconstructed phylogeny and geographical history of the species (Coleoptera: Cicindelidae). Quaestiones Entomologicae 24: 51-161.

Spence, J.R. 1980. Density estimation for water-striders. Freshwater Biology 10: 563-570.

Spence, J.R. 1981. Experimental analysis of microhabitat selection in water-striders (Heteroptera: Gerridae). Ecology 62: 1505-1514.

Spence, J.R. 1983. Pattern and process in co-existence of water-striders (Heteroptera: Gerridae). Journal of Animal Ecology 52: 497-511.

Spence, J.R. 1986. Interactions between the scelionid egg parasitoid *Tiphodytes gerriphagus* (Hymenoptera) and its gerrid hosts (Heteroptera). Canadian Journal of Zoology 64: 2728-2738.

Spence, J.R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Canadian Journal of Zoology 68: 1770-1782.

Spence, J.R. 2000. Seasonal aspects of flight in water striders (Hemiptera: Gerridae). Entomological Science 3: 399-417.

Spence, J.R. and Wilcox, R.S. 1986. The mating system of two hybridizing species of water striders (Heteroptera: Gerridae). II. Alternative tactics of males and females. Behavioural Ecology and Sociobiology 19: 87-95.

Spence, J.R. amd Scudder, G.G.E. 1980. Habitats, life cycles, and guild structure among water striders (Heteroptera: Gerridae) on the Fraser Plateau of British Columbia. Canadian Entomologist 112: 779-792.

Sperling, F.A.H. 1987. Evolution of the *Papilio machaon* species group in western Canada. Quaestiones Entomologicae 23: 198-315.

Sperling, F.A.H. 1990. Natural hybrids of *Papilio* (Insecta: Lepidoptera): Poor taxonomy or interesting evolutionary problem? Canadian Journal of Zoology 68: 1790-1799.

Sperling, F.A.H. and Spence, J.R. 1990. Allozyme survey and relationships of *Limnoporus* Stål species (Heteroptera: Gerridae). Canadian Entomologist 122: 29-42.

Sperling, F.A.H. and Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370-1383.

Wang, H., Byrd, D.W., Howard, J.L., McArthur, E.D., Graham, J.H. and Freeman, D.C. 1998. Narrow hybrid zone between two subspecies of big sagebrush (*Atermisia tridentata*: Asteraceae). V. Soil properties. International Journal of Plant Science 159: 139-147.

Wang, H., McArthur, E.D., Sanderson, S.C., Graham, J.H., and Freeman, D.C. 1997. Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae). IV. Reciprocal transplant experiments. Evolution 51: 95-102.

GENERAL DISCUSSION

Understanding how hybridization influences populations of interbreeding species, in terms of morphology and genetics, are the central questions of this thesis. Previous studies on a diverse array of hybridizing taxa including plants, insects, fish and mammals (see Arnold 1997, Harrison 1993), make it apparent that the answer to this question will vary with the biology of the organisms involved, as well as the ecological and historical context of hybridization. Chapters 2 to 3 of this thesis improve our understanding of the consequences of hybridization on morphology and genotype in *Limnoporus* water striders. This work also helps define the geographical boundaries of introgression. Chapter 4 points to an environmental influence on population density as an important factor shaping the patchy population structure of this hybrid zone (Spence 1990, Sperling and Spence 1991). Predicting long-term consequences of natural hybridization on evolution and conservation of species populations requires knowledge of the effect of hybridization on species, and an understanding of the factors influencing hybrid zone dynamics.

Genetic consequences of hybridization

Genomes of *L. dissortis* and *L. notabilis* appear relatively impermeable to introgression by a mitochondrial marker, CO1. However, more variation is shared among species at the nuclear loci, EF1-α and ITS 1. In particular, there is extensive introgression at the ITS 1 locus, suggesting that this locus may undergo neutral or positive selection in the hybrid zone. These results support the idea that introgression is selective (Harrison 1986, Martinsen et al. 2001), and that hybrid zones may act as "evolutionary filters."

Whether or not introgression will lead to adaptive evolution in *Limnoporus* species is not certain, and remains an open question in hybrid zone studies (Arnold 1997).

That mitochondrial DNA appears to be a good marker of species boundaries for *Limnoporus* is an uncommon exception to Haldane's Rule (1922). Under this hypothesis, species boundaries should correspond with mtDNA markers for taxa with heterogametic females, such as Lepidoptera (Sperling 1993, Sperling 1994, Prowell 1998) and birds (Tegelström and Gelter 1990). *Limnoporus* hybrids are inconsistent with Haldane's Rule because females are selected against (Spence 1990, Sperling and Spence 1991) but are the homozygous sex (Spence and Maddison 1986).

Selection against female hybrids

Demonstrations of strong selection against F_1 females (Spence 1990, Sperling et al. 1997) raised the hypothesis that genetic incompatabilities in female hybrids have a strong influence on the structure of the *Limnoporus* hybrid zone. A strong barrier to mtDNA introgression, shown in Chapter 2, is consistent with a hypothesis of selection against F_1 female hybrids. However, females resulting from backcrossing may be relatively common. In Chapter 3, morphological analysis of natural populations reveals that hybridized females and males are equally common in the southwestern region of Alberta, suggesting that most 'hybrids' here result from backcrossing. Thus, an important role of backcrossed females for facilitating introgression may apply to regions of the *Limnoporus* hybrid zone where F_1 hybrids are rare. Relative abundance of F_1 and backcrossed hybrids

varies by taxa (Jiggins and Mallet 2000), and may vary regionally within the *Limnoporus* hybrid zone.

More questions about the mechanisms causing incompatability in some hybrid females but not others emerged from this thesis. A lack of female F1 hybrids that could be raised in the laboratory, prompted Spence (1990) to hypothesize low fitness in females is due to either incompatible X chromosomes, or incompatible nuclear and cytoplasmic genomes. Sex-linked genes seem to be little involved in regulation of growth because reciprocal F1 males do not appear distinguished in multivariate morphometric space. However, a test of sex-linked effects that compares differences in reciprocal F1 hybrids between males and females, is not possible for *Limnoporus* hybrids because F1 females fail to develop (Spence 1990). Sperling et al. (1997) hypothesized that a factor causing developmental failure in interspecific crosses with other *Limnoporus* species is an autapomorphy within *L. dissortis*. Co-regulation of mitochondrial and nuclear genomes might be necessary for normal development and requires further investigation in *Limnoporus* hybrids.

Environmental influence on hybridization

Mosaic nature of habitats

Regional variation in the genetic structure of the *Limnoporus* hybrid zone may be accounted for by temporary *Limnoporus* habitats that are patchy in both space and time (Spence 1989). Because water striders require aquatic habitats to breed, their movement between habitats is limited to areas with suitable habitats that are within dispersing distance of each other. Anthroprogenic disturbance, like road construction or agricultural

activity in western Canada, tends to be patchily distributed within the environment and appears to expand the number of habitats available. *Limnoporus* habitats that are formed and lost in an unpredictable way create fluctuations in population size and dispersal. The temporary and patchy nature of *Limnoporus* habitats exerts a strong influence on patterns of dispersal and gene flow.

Selective pressure for frequent, active dispersal in *Limnoporus* may result from many factors. Strong selection for disperal to temporary habitats results from extremely high rates of egg parasitism by the wasp *Tipodytes gerriphagus* (Marchal) in permanent ponds (Spence 1986). In temporary or newly created habitats, rates of parasitism are relatively low compared to parasitism rates exceeding 95% for some *L. dissortis* populations (Spence 1986). However, dispersal to more permanent habitats later in the summer is favoured to avoid laying eggs in a habitat that will dry out, and to spread reproductive risk over several habitats (Spence 2000). Thus, strong selection pressure for frequent dispersal into new habitats can cause considerable mixing of populations that are within dispersing distance of each other.

Location of hybridized populations

Patchy population densities, combined with extinction and colonization, can broaden hybrid zones (Butlin et al. 1991), although this effect depends on the scale of patchiness. Large patch sizes may concentrate differences between species at the patch barrier. The region I sampled in south-eastern British Columbia appears to act as one large patch that is characterized by few suitable habitats. Lack of suitable habitats may

also explain why hybrids are concentrated along the eastern foothills in south-western Alberta. Smaller patch sizes in sparsely populated regions may show more introgression than elsewhere, and broaden clines (Hewitt 1989). This may apply to the region along Highway 16 through Jasper National Park where habitats appear to be ecologically marginal, and account for a broader expanse of hybridzation into British Columbia. Our ability to predict regions where hybrids will occur may improve with an understanding of how population density varies regionally across the *Limnoporus* hybrid zone. Over time, long term climate changes that significantly alter the availability and/or suitability of habitats may impact the dynamics of this hybrid zone.

Mountain passes

Along the east slopes, introgression is most extensive in regions where a highway has been constructed through the Rocky Mountains; along Highway 16, through Jasper National Park, and south of Highway 1 near Calgary. In contrast, in regions where the highway along the east slopes was not connected by a road through the mountains, such as Highway 40 north and south of Hinton, I found very few hybrids (Chapter 3). The importance of mountain passes in facilitating hybridization between species divided by mountains was emphasized by Remington (1968) in his description of the Pacific-Rocky Mountain "suture zone". He noted that "suturing" or hybridizing within this region, was concentrated near mountain passes. In addition to passes ameliorating altitudinal barriers, highway construction likely increases the availability of *Limnoporus* habitats created through disturbance, like roadside ditches. Patterns of morphometric and genetic variation

(Spence 1990, Sperling and Spence 1991, Chapter 2) on either side of the moutain pass raise the hypothesis that mountain passes act as a conduit for *dissortis*-like hybrids to disperse into *notabilis*-like populations on the west side of the mountains.

Why introgression does not occur in the reverse direction, from *L. notabilis* eastward into *L. dissortis* populations, is not clear. One possibility is that *L. dissortis* habitats east of the foothills in Alberta tend to be less permanent than *L. notabilis* habitats west of the mountains. Permanence of habitats may influence the direction of gene flow if relatively permanent habitats facilitate movement of *L. dissortis* westward, but temporary habitats east of the mountains present limitations to the eastward movment of *L. notabilis.*

Asymmetrical mating success

Asymmetrical introgression (Spence 1990, Sperling and Spence 1991, Chapters 2 and 4), from *L. dissortis* populations into *L. notabilis* populations, may be explained by close examination of the mating system. Although results of cytonuclear disequilibria are inconclusive about unidirectional matings among *Limnoporus*, the common occurence of unidirectional hybridization in other taxa emphasizes the importance of sexual selection in promoting hybridization for only certain combinations of interspecific matings (Wirtz 1999). *Limnoporus* males can display alternative mating tactics (territorial signallers, patrolling signalers and silent patrollers) and in mixed populations, choice of mating tactic is associated with body size (Spence and Wilcox 1986). Thus, asymmetrical interspecific mating success among males may be attributed to the greater success of sneak copulations by smaller-bodied males, which are mainly *L. dissortis* or hybrids, as compared to the

strategy of sending precopulatory ripple signals, as employed by larger-bodied males, which are mainly *L. notabilis* (Wilcox and Spence 1986).

Female resistance to interspecific copulations may also influence the success of *L. dissortis* males compared to *L. notabilis* males. For water striders, males are generally indiscriminate in their mating efforts (Arnqvist 1997). Female choosiness is mediated by various precopulatory struggles, which dislodge undesirable males (Spence and Andersen 1994). For *L. notabilis* and *L. dissortis*, physical struggles between sexes have not been reported, but females will show reluctance to mate when courted by a signalling male, and generally terminate matings (Spence and Wilcox 1986). Furthermore, a behavioural signal given by *L. notabilis* females may be an important component of courtship (Spence and Wilcox 1986). Because copulation duration can last up to 25 minutes for *Limnoporus* species (Spence 1990), costs of mating for females, such as increased predation risk or energy expenditure (Arnqvist 1997) may increase in matings with a relatively large-bodied male. Thus, *L. notabilis* females may be more willing to accept mating attempts from a smaller-bodied *L. dissortis* or hybrid male compared to the reciprocal combination.

However, when number of conspecific males is limited, females of both species may be more willing to accept a copulation from an heterospecific male rather than forgo mating altogether. For example, early colonizers to a newly formed habitat certainly experience limited mate choices when the number of colonizers is small. Compared to other gerrids, *Limnoporus* populations are generally lower (Spence and Scudder 1980). Thus, for habitats located within the transition zone between species, low population

density may reduce levels of mate discrimination and elevate amounts of interspecific mating.

Future considerations

Future work on the *Limnoporus* hybrid zone can be extended in several directions. Experimental studies of mating behaviour can test assumptions about the influence of the ratio of conspecific verus heterospecific mates on mate choice. Such experiments would establish a condition-dependent behavioural component of hybridization, and emphasize the importance of fluctuating population size on mating decisions.

The possibility that variation in population density across the hybrid zone contributes to a patchy genetic structure requires further studies. Now that the hybrid zone has been roughly defined in terms of regional habitat availability, future work may develop a detailed comparison of patterns of gene flow in relation to *Limnoporus* abundance, habitat quality and habitat availability, across several transects, including samples in central British Columbia. Thus, predictions about patterns of introgression can be made if patchy population structure has the effect of broadening clines in regions of low population density and shallow density gradients (Nichols and Hewitt 1986, Nichols 1989), and concentrating clines at barriers to dispersal, where population density would be at a low extreme (Butlin et al. 1991).

Although this work suggests that active species preferences for different habitats are not an important component of *Limnoporus* hybrid zone dynamics, a better way to assess selection for different habitat types is to conduct reciprocal transplant experiments of different genotypes into natural habitats located across the hybrid zone. So far, this

approach has been limited to sessile organisms (e.g. plants: Emms and Arnold 1997, Campbell and Waser 2001, clams: Arnold et al. 1996) although it could also be applied to *Limnoporus*.

The genetic mechanism that contributes to the reproductive isolation between these species remains unknown. mtDNA may not act as a neutral marker in the *Limnoporus* hybrid zone. One way to assess if nuclear-mitochondrial genomic interactions are responsible for misregulating development in hybrid females (Spence 1990) is to measure development and hatching success in laboratory crosses designed to increase the disparity between mitochondrial and nuclear genomes. Under this hypothesis, increased species disparity between nuclear and mitochondrial genomes would result in increased developmental failure. Dawson et al. (1993) conducted such an experiment with hybridizing mice, producing 5 generations of successive backcrosses, while maintaining the mitochondrial lineage and increasing the contribution of nuclear genes from the other species. This experimental design cannot distinguish between nuclear-cytoplasmic effects and nuclear-mitochondrial interactions. Studies designed to distinguish between cytoplasmic and mtDNA effects are possible using a technique of replacing mtDNAs between strains by microinjections (e.g. Niki et al. 1989). More experimental work is necessary to understand the genetic basis of nuclear-mitochondrial fitness interactions.

In summary, this thesis suggests that future work can most profitably explore the interactions of environment and genetics that influence patterns of introgression in this hybrid zone, and the genetic mechanisms that constrain it. The morphometric and genetic

tools developed here provide a basis for identifying hybridized populations, and descriptions of regional patterns of introgression and habitat availability can be used to improve sampling design and facilitate hypothesis testing in future studies. Future researchers of the *Limnoporus* hybrid zone will find tremendous opportunties here for new contributions to our understanding of hybrid zone processes.

REFERENCES

Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York, New York.

Arnold, W.S., Bert, T.M., Marelli, D.C., Cruz-Lopez, H. and Gill, P.A. 1996. Genotype-specific growth of hard clams (genus *Mercenaria*) in a hybrid zone: variation among habitats. Marine Biology 125: 129-139.

Arnqvist, G. 1997. The evolution of water strider mating systems: causes and consequences of sexual conflicts. Pp. 146-163 *in* J.C. Choe and B.J. Crespi, eds. Mating Systems in Insects and Arachnids. Cambridge University Press, New York, New York.

Butlin, R.K., Ritchie, M.G., and Hewitt, G.M. 1991. Comparisons among morphological characters and between localities in the *Chorthippus parallelus* hybrid zone (Orthoptera: Acrididae). Philosophical Transactions of the Royal Society of London, Series B 334:297-308.

Campbell, D.R. and Waser, N.M. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55: 669-76

Dawson, W.D., Sagedy, M.N., En-yu, L., Kass, D.H. and Crossland, J.P. 1993. Growth regulation in *Peromyscus* species hybrids: a test for mitochondrial-nuclear genomic interaction. Growth, Development and Aging 57: 121-133.

Emms, S.K. and Arnold, M.L. 1997. The effect of habitat on parental and hybrid fitness transplant experiments with Louisiana irises. Evolution 51: 1112-1119.

Haldane, J.B.S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12: 101-109.

Harrison, R.G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56: 337-349.

Harrison, R.G. 1993. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York, New York.

Hewitt, G.M. 1989. The fragmentation of tension zones in sparsely populated areas. The American Naturalist 134: 969-977.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

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

Martinsen, G.D., Whitham, T.G., Turek, R.J., and Keim, P. 2001. Hybrid populations selectively filter gene introgression between species. Evolution 55: 1325-1335.

Nichols, R.A. and Hewitt, G.M. 1986. Population structure and the shape of a chromosomal cline between two races of *Podisma pedestris* (Orthoptera: Acrididae). Biological Journal of the Linnean Society 29: 301-316.

Nichols, R.A. 1989. The fragmentation of tension zones in sparsely populated areas. The American Naturalist. 134: 969-977.

Niki, Y., Chisgusa, S.I., and Matsuura, E.T. 1989. Complete replacement of mitochondrial DNA in *Drosophila.* Nature 341: 551-552.

Prowell, D.P. 1998. Sex linkage and speciation in Lepidoptera. Pp. 309-319 *in* D.J. Howard and S.H. Berlocher, eds. Endless Forms, Species and Speciation. Oxford University Press, New York.

Remington, C.H. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evolutionary Biology 2: 321-428.

Spence, J.R. 1986. Interactions between the scelionid egg parasitoid *Tiphodytes gerriphagus* (Hymenoptera) and its gerrid hosts (Heteroptera). Canadian Journal of Zoology 64: 2728-2738.

Spence, J.R. 1989. The habitat templet and life history strategies of pond skaters (Heteroptera: Gerridae): reproductive potential, phenology, and wing dimorphism. Canadian Journal of Zoology 67: 2432-2447.

Spence, J.R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Canadian Journal of Zoology 68: 1770-1782.

Spence, J.R. 2000. Seasonal aspects of flight in water striders (Hemiptera: Gerridae). Entomological Science 3: 399-417.

Spence, J.R. and Andersen, N.M. 1992. Classification and phylogeny of the Holarctic water strider genus *Limnoporus* Stål (Hemiptera, Gerridae). Canadian Journal of Zoology 70: 753-785.

Spence, J.R. and Andersen, N.M. 1994. Biology of water striders: Interactions between systematics and ecology. Annual Review of Entomology 39: 101-128.

Spence, J.R. and Maddison, D.R. 1986. Chromosomes of two hybridizing species of *Limnoporus* (Heteroptera: Gerridae). Proceedings of the Entomological Society of Washington 88: 502-508.

Spence, J.R. and Scudder, G.G.E. 1980. Habitats, life cycles, and guild structure among water striders (Heteroptera: Gerridae) on the Fraser Plateau of British Columbia. Canadian Entomologist 112: 779-792.

Spence, J.R. and Wilcox, R.S. 1986. The mating system of two hybridizing species of water striders (Gerridae) II. Alternative tactics of males and females. Behavioural Ecology and Sociobiology. 19: 87-95.

Sperling, F.A.H. 1993. Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. Heredity 71: 227-233.

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

Sperling, F.A.H. and Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370-1383.

Sperling, F.A.H., Spence, J.R. and Andersen, N.M. 1997. Mitochondrial DNA, allozymes, morphology and hybrid compatability in *Limnoporus* water striders (Heteroptera: Gerridae): Do they all track species phylogenies? Annals of the Entomological Society of America 90: 401-415.

Tegelström, H. and Gelter, H.P. 1990. Haldane's rule and sex-biased gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). Evolution 44: 2012-2021.

Wilcox, R.S. and Spence, J.R. 1986. The mating system of two hybridizing species of water striders (Gerridae). Behavioural Ecology and Sociobiology 19: 79-85.

Wirtz, P. 1999. Mother species-father species: unidirectional hybridization in animals with female choice. Animal Behaviour 58: 1-12.

APPENDIX: ADDITIONAL INFORMATION ABOUT SAMPLE SITE LOCATIONS

Chapter 1

Locality #	Location
0	Marshy habitat with many grassy inlets and patches of open water. Just west of Niton Junction, on the north side of Highway 16.
1	Includes specimens from 2 closely spaced sampling locations. The first one is a long ditch about 2.5 km west of Edson along Hwy 16, located under a billboard that advertises "White Water Rafting." The second location is about 6.5 km west of Edson on the north side of Hwy 16. It is a pond with a small sign "Warning High Pressure Gas Pipeline-Nova".
2	A long ditch on the north side of the Hwy 16 near Galloway Road, with a large "Galloway Road" sign in front of the ditch. About 22.1 km from locality #1 (the one 6.5 km west of Edson).
3	A shallow water body located on the north side of Hwy 16. Located beside a pullout; just before the pullout is a "Do not enter" sign and a "One way" sign. Located just east of a large body of water. About 14 km west of locality #2.
4	North side of Hwy 16, in a clearing behind a long guard rail. About 8.6 km west of locality #3.
5	Located on north side of Hwy 16, as you enter Hinton town limits from the east. Located under "Dairy Queen" and "Subway" billboards. About 25 km west of locality #4.
6	Located on the north side of Hwy 16, across from farms and ranches. About 13.1 km from locality #5.
7	Includes specimens from 2 closely spaced sample locations inside Jasper National Park along Hwy 16. The first location is about 9 km west of the gates entering Jasper National Park from the east. It is across from Roche Miette on the north side of the highway and looks like the backwaters of a large body of water. There is a beaver lodge here. The second location is a large body of water about 1.9 km west of the first one, and is also on the north side of the highway.

8	About 27 km west of the British Columbia/Alberta border, going east on Hwy 16. It is on the north side of the road, just after Mt. Mowat.
9	Marion Lake, The Malcolm Knapp Research Forest (University of British Columbia), Maple Ridge, British Columbia

Chapter 3

Locality	Location
1	A ditch along Hwy 16, on the west side of the road. About 62 km north of McBride and past the towns of Crescent Spur and Loos, B.C. Just before Catfish Creek. On a curve of the road with an empty field across the road.
2	Located less than a km from locality #3, along Hwy 16.
3	Located along Hwy 16, about 13 km north of McBride, B.C, in front of a clear cut patch and a pile of logs
4	Located along Hwy 5, on the west side of the road. About 43 km south of Valemount, B.C. Access down a slight slope from the road. Behind the pond is a mountain with a rectangular clear cut in the background.
5	Along Hwy 5, on the west side of the road. Near a sign "Entering Blue River Improvement District." Near Cook Creek. Dead standing tress nearby.
6	Along Hwy 5, south of Blue River on the east side of the road. 3 km south of a "Little Hell's Gate" sign. Access is down a slope from the road. There is a clear cut and a barren hill behind the site and some standing dead trees. There is an empty grassy patch near the pond (I refer to this as the "Landing Pad")
7	Near the end of Hwy 23, near Mica Dam on the west side of the road. There is a small road leading down to the pond and a sign indicating the road is closed. There are many large stumps in the pond.
8	Along Hwy 23, on the east side of the road. There is a creek trickling into a ditch, with power lines overhead and a rocky ridge on the opposite side of the road. 6 km south of locality # 9.

9	Along Hwy 23, about 68 km north of the junction with Hwy 1 (east). A ditch on the east side of the road, fed by a slight waterfall. Lake Revelstoke is on the opposite side of the road. There is a big pile of wood in the middle of this site.
10	Inside Revelstoke town limits, along Hwy 23 on the west side of the road. This is a ditch at the bottom of a rock wall, opposite a church and next to a gas station by Maley Road.
11	Along Hwy 95, south of Golden and about 4 km north of Parson, B.C. Traveling south, there is a blue farmhouse on the left and a railroad on the right side of the road.
12	West of the BC/AB border on north side of Hwy 16, just past Mt. Mowat.
13	Along Hwy 40, north of Grande Cache. Near a sign "Musreau Lake 6 km" and a sign "Grande Prairie 70 km."
14	Along Hwy 40, on the east side of the road. About 106 km south of Grande Prairie. There is a "no poaching" sign nearby, and it is located on a curve in the road.
15	Along Hwy 47, on the west side of the road. About 14 km north of the junction with Hwy 40 (Forestry Trunk Road), north of Robb, AB. The ditch is located in front of a regenerating forest.
16	Along Hwy 47, on the east side of the road. About 1 km north of the junction with Hwy 40 (Forestry Trunk Road). There is a railroad nearby, and the Embarras River is just down the road (going north).
17	Along Hwy 734 (Hwy 40, Forestry Trunk Road), on west side of road. About 44 km south of the junction with Hwy 47. Dead standing trees in and surrounding the pond.
18	Shallow ditch along the Hwy 734 on the east side of the road. About 27 km north of the junction with Hwy 11 (near Nordegg).
19	Along Hwy 734, on east side of road. About 8 km south of the junction with Hwy 591. Access is down a steep rocky slope. 6 km south of a blue bridge over the Clearwater River.

20	Along Hwy 40, on right (east?) side of road traveling north, west of Cochrane. Three is a house with horse stables on the opposite side of the road. Still stream runs through a culvert with some caging on it.
21	Along secondary Hwy 762, on east side of road. About 12 km north of the junction with secondary Hwy 549. Winding still stream with many inlets and beaver dams. Traveling north, there is a"Vineripe Green houses" just past turnoff to this site, and a large body of water with a dock just before the turnoff.
22	Along Hwy 22, on the east side of the road. About 25 km north of the junction with Hwy 541. Surrounded by cow pasture.
23	Along Hwy 22, on the west side of the road. About 7 km north of the junction with Hwy 541. There is a large culvert next to the pond, and a small blue house across the road. Traveling north, it is just before Tongue Creek Road sign and an Info Centre Sign.
24	Along secondary Hwy 541, about 30 km east of the junction with Hwy 40. Traveling east, you pass a "Welcome to the Foothills" sign, and a ranch with antler gates on the right. The pond is beside a large wooden gate.
25	Along secondary Hwy 940 (Forestry Trunk Road), on the left side of the road (traveling north). About 28 km north of the Dutch Creek campsite, just before a "Husky Oil trucks chain up here" sign.
26	Along secondary Hwy 940 (Forestry Trunk Road). About 18 km north of junction with Hwy 3. Pond is in front of a hill, with not many trees surrounding it.
27	Along Hwy 3, just east of Coleman. Access to the site is via an access road that parallels the highway. The stream winds through an old orchard, it is beside train tracks and a lumber yard.
28	Along Hwy 6, on the west side of the road. About 18 km south of Pincher Creek. Just past a black bridge covered in graffiti. Slow stream surrounded by cow pasture.
29	Along secondary Hwy 505, on the north side of the road. 32 km west of the junction with Hwy 2. The ditch is in front of a very new big home on the north side of the road and is just west of a large body of water.

30	Along Hwy 16 about 43 km west of Edson on north side of road. Located beside a pullout; just before the pullout is a "Do not enter" sign and a "One way" sign. Located just east of a large body of water.
31	Along Hwy 16, about 14 km east of locality # 30. A long ditch on the north side of the Hwy 16 near Galloway Road, with a large "Galloway Road" sign in front of the ditch.
32	Along Hwy 16, about 25 km west of Edson on north side of road. It is just before a "curve" sign. It is a slow narrow stream behind a tall barbed wire fence, surrounded by trees and shrubs.
33	Along Hwy 16, just west of Niton Junction. Marshy habitat with many grassy inlets and patches of open water.
34	Along Hwy 16, west of Wildwood. A train track runs right above the pond.
35	Along Hwy 36, south of Two Hills, on the east side of the road. About 5 km north of Twp Rd 540.
36	Along Hwy 36, south of Two Hills, on the east side of the road. About 7 km north of Twp Rd 540.
37	Along secondary Hwy 881, north of St. Paul, on the west side of the road. 1 km north of Twp Rd 584, just past "Canada Life."
38	Along secondary Hwy 659, east of Bonnyville, on the south side of the road. There is a beaver dam here, and "Murphy Livestock" is across the road.
39	Along secondary Hwy 897, 14 km north of Frog Lake, on the west side of the road. Marshy site with a fence in front of it.
40	Across the road from locality #39. Surrounded by trees.