# Molecular and morphological divergence in the butterfly genus *Lycaeides* (Lepidoptera: Lycaenidae) in North America: evidence of recent speciation

C. C. NICE*† & A. M. SHAPIRO*

*Section of Evolution and Ecology and Center for Population Biology, University of California, Davis, CA 95616, USA
†White Mountain Research Station, University of California, San Diego, La Jolla, CA 92093, USA

*Keywords:*

allozymes;
biogeography;
*Lycaeides*;
mitochondrial DNA;
morphology;
speciation.

## Abstract

Male genital morphology, allozyme allele frequencies and mtDNA sequence variation were surveyed in the butterfly species *Lycaeides idas* and *L. melissa* from across much of their range in North America. Despite clear differences in male genital morphology, wing colour patterns and habitat characteristics, genetic variation was not taxonomically or geographically structured and the species were not identifiable by either genetic data set. Genetic distances (Nei's $D = 0.002$–$0.078$, calculated from allozyme data) between all populations of both species were within the range commonly observed for conspecific populations of other butterflies. The most frequent mtDNA haplotype was present in individuals of both species in populations from southern California to Wisconsin. We conclude that speciation has probably happened recently and the lack of genetic differentiation between the species is the product of either (1) recent or ongoing gene flow at neutral loci, and/or (2) an insufficiency of time for lineage sorting. The evolution of male genital morphology, wing colour patterns and ecological characteristics has proceeded more rapidly than allozyme or mtDNA evolution.

## Introduction

*Lycaeides idas* (Linnaeus) and *L. melissa* (W. H. Edwards) (Lepidoptera: Lycaenidae) are broadly sympatric in several regions of North America (Fig. 1) and are morphologically distinguishable by qualitative wing pattern differences (Tilden & Smith, 1986), quantitative differences in male genital morphology (Nabokov, 1949; Lane & Weller, 1994) and by ecological differences in habitat specificity and hostplant associations (Ferris & Brown, 1981; Garth & Tilden, 1986; Scott, 1986; Tilden & Smith, 1986; Opler, 1992). Based on these characteristics, these species are further subdivided currently into 17 subspecies (12 in *L. idas*, five in *L. melissa*) (Miller & Brown, 1981; Ferris, 1989), two of which have attracted the attention of conservation and evolutionary biologists: *L. m. samuelis*, the Karner Blue butterfly, is a federally endangered subspecies (US Fish and Wildlife Service, 1992; Lane, 1994); *L. i. lotis*, the Lotis Blue, has the same status but is now believed to be extinct (Arnold, 1993). Nabokov (1949) suggested that limited hybridization between *L. idas* and *L. melissa* may occur in isolated localities. In particular, populations in the Warner Mountains of north-eastern California may be of hybrid origin. We studied the evolutionary relationships among populations of these two species and investigated the hypothesis of hybridization by surveying variation in morphology and in two kinds of genetic markers.

The entity we are calling *idas* was separated on genitalic grounds from the palearctic species *L. argyrognomon* by Higgins (1985) but is often referred to under that name in the older Nearctic literature. As currently defined, *Lycaeides idas* is a circumpolar, Holarctic, polytypic species while *L. melissa* is purely North American.

*Correspondence:* Chris C. Nice, Department of Entomology, 840 Russell Labs, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA.
Tel: +1 608 265 2470; fax: +1 608 262 3322;
e-mail: ccnice@facstaff.wiscedu

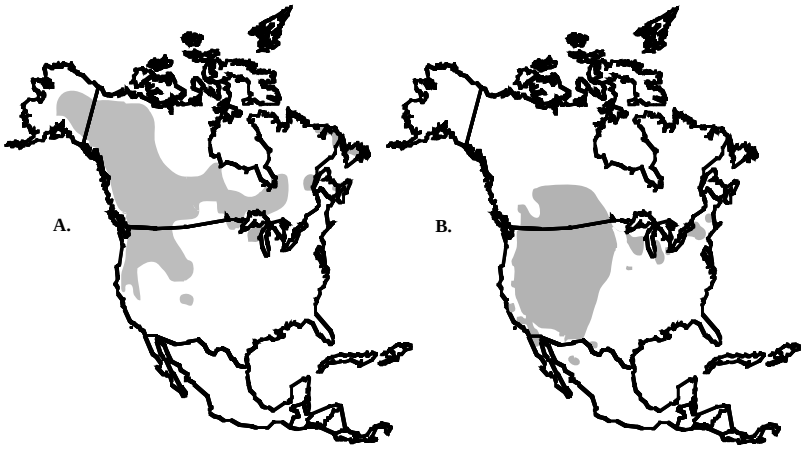


**Fig. 1** The approximate ranges of (A) *Lycaeides idas* and (B) *L. melissa* in North America (following Nabokov, 1949; Opler, 1992; Stanford & Opler, 1996; Scott, 1986). The present range of *L. melissa* in eastern North America is very fragmented; this figure illustrates the historical range of this butterfly.

Both species feed as larvae on papilionaceous legumes (Fabaceae) and *L. idas* populations in the north-eastern part of its range also use *Empetrum nigrum* (Empetraceae) and several members of the Ericaceae (Garth & Tilden, 1986; Scott, 1986; Opler, 1992). Nearctic populations assigned to *L. idas* by habitus and genital morphology are all univoltine and most occur in moist or wet environments including streamsides, fens, bogs and swamps. Populations of *L. melissa* associated with native hostplants are also univoltine but often occur in drier grassland, steppe or alpine environments, the only exception being populations of *L. m. samuelis* (the Karner Blue Butterfly), which is bivoltine. Throughout most of western North America there are also populations of *L. melissa* which feed on cultivated alfalfa, *Medicago sativa*, and are two- or three-brooded. Alfalfa was introduced in western North America and probably arrived with immigrants to California around 1850 (J. Gerlach, personal communication). In many areas, *L. melissa* has spread to escaped roadside alfalfa but rarely, if ever, uses native hosts in such situations. These populations are presumably of recent origin; at least no multivoltine populations have been found in the absence of alfalfa over nearly all of this vast range, a situation akin to that found in *Papilio zelicaon* Lucas (Lepidoptera, Papilionidae) feeding on naturalized *Foeniculum* in California (Shapiro, 1995). Populations of both species, representing several named subspecies, are interdigitated in California and are normally easy to differentiate, regardless of geography.

The presumed extinct Lotis Blue was unusual in that its genital morphology and habitat (bogs along the northern California coast) were typical of the taxon *idas*, while its wing phenotype was much more typical of *melissa*. There is an extant allopatric group of populations of uncertain placement which Scott (1986) considered to be *lotis* as well. These are located in the Warner Mountains of extreme north-eastern California (and are referred to as the 'Warners entity' hereafter). They are relatively abundant in a high montane Great Basin environment and display considerable variation, but basically present *idas*-like genitalia and larval primary setation (Ballmer & Pratt, 1988) with a *melissa* habitus and habitat. We suspected that these populations – true *lotis* and the Warners entity – might have been of hybrid origin and undertook genetic studies in the expectation that such good 'morphospecies' as *idas* and *melissa* would have easily detected differences which we could then use to test the hybrid swarm hypothesis. Nabokov (1949) recognized a possibly similar situation in north-western Wyoming and suggested that *L. i. longinus* might be of hybrid origin, but no subsequent studies have been done.

We surveyed morphological variation in male genitalia in population samples from across much of North America following the work of Nabokov (1949) to confirm the morphological basis for distinguishing *idas* and *melissa*. These morphological patterns were compared to patterns of geographical genetic structure obtained by surveying allozyme electrophoretic variation in populations of both species from California and western Nevada. We compared the allozyme survey results to mitochondrial DNA (mtDNA) sequence variation in both species across much of the continental United States.

## Materials and methods

### Genitalic morphology

The posterior-most abdominal segments of 311 male *Lycaeides* were removed from pinned specimens and from specimens that were subsequently processed for allozyme surveys and mtDNA extraction. These specimens were identified using wing phenotypes and included 96 *L. idas*, 186 *L. melissa* and 29 males from the Warner Mountains entity. Appendix A provides locality and sample size data. Pinned specimens were labelled and are stored as vouchers. The abdomens of females and the wings of all individuals processed for morphological and genetic analyses were retained and stored in labelled glassine envelopes as vouchers.

After submersion in hot (≈100 °C) 5 M KOH, male abdomens were dissected and genitalia removed under a dissecting microscope. Each specimen was positioned under a cover slip such that the valvae were pushed out of the way and the right half of the uncus and the right falx could be viewed from the ventral side. Using an ocular micrometer, five measurements were taken as described by Nabokov (1949) (Fig. 2). One-half of the individuals from each population of *L. idas* and *L. melissa* were selected and discriminant analysis (Snedecor & Cochran, 1967) using the JMP Version 3.15 statistical software (SAS Institute, Cary, NC, USA, 1994) was performed using these five measurements to assess the morphological separation of the two species. Multivariate analyses of variance were also performed to assess the contributions of each of the five measurements using a discriminant analysis program of Ludwig & Reynolds (1988) as modified by Rejmánek & Richardson (1996). The best combination of measurements was then used to classify individuals. The accuracy of this classification was assessed by 100 cross-validation runs where half of the individuals of *L. idas* and *L. melissa* were randomly chosen each time. The discriminant function was then used to classify individuals from the Warner Mountains populations, which had not been used in its development. Discriminant analysis was also performed treating the Warner Mountains populations as a third 'species'.

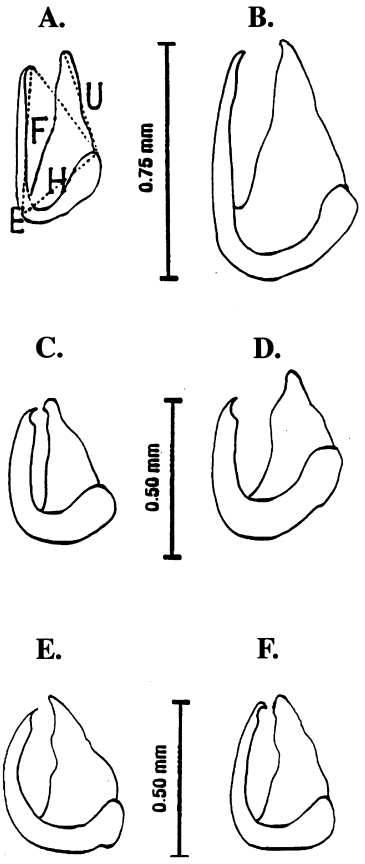


**Fig. 2** Male genitalia illustrated. (A) The five measurements: F = falx length, H = humerulus length, FH = falx to humerulus distance (the distance from the distal end of the falx to the distal end of the humerulus at its intersection with the uncus), U = uncus length, E = elbow width (following Nabokov, 1949). (B) Specimen of *L. melissa* from Gazelle, Siskiyou County, CA. (C) Specimen of *L. idas* from Indian Valley, Trinity County, CA. (D) Specimen of *L. idas* from Leek Springs, El Dorado County, CA. (E,F) Two specimens from Emerson Peak, Warner Mountains, Modoc County, CA. Illustrations by L. Ellis.

## Allozyme methods

Individuals of both sexes ($n = 264$) from 14 populations were collected for starch gel allozyme electrophoresis. Appendix B provides locality and sample size data for these specimens and Fig. 3 illustrates their locations. Specimens were captured and maintained alive until frozen in either a –80 °C freezer at the University of California, Davis, or in a liquid nitrogen thermos in the field. Those frozen in liquid nitrogen were transferred to –80 °C freezers upon arrival at UC Davis. Wings and the posterior portions of the abdomens (for males) or the whole abdomens (for females) were removed and stored in numbered and dated glassine envelopes inscribed with locality and date of capture data. (Male genitalia were later removed for morphological study, see above.) The remaining bodies of each individual were placed in a 0.5-mL microcentrifuge tube and prepared for allozyme analysis in Dr H. B. Shaffer's laboratory at UC Davis as described in Porter & Mattoon (1989).

Twenty-four presumptive loci were surveyed, of which 10 were polymorphic and reliably scorable. These polymorphic loci, listed with their abbreviations and their IUBNC Enzyme Commission numbers (Shaklee *et al.*, 1990), are: adenylate kinase (AK) (2.7.4.3), fructose-bisphosphate aldolase (FBA) (4.2.1.13), glucose-6-phosphate isomerase (GPI) (5.3.1.9), glycerol-3-phosphate dehydrogenase (G3PDH) (1.1.1.8), hexokinase (two loci: HK-1, HK-2) (2.7.1.1), isocitrate dehydrogenase (two loci: IDH-1, IDH-2) (1.1.1.42), malate dehydrogenase (MDH) (1.1.1.37) and phosphoglucomutase (PGM) (5.4.2.2). Electromorphs were given letter designations arbitrarily. Potentially ambiguous electromorphs were rerun in adjacent lanes to confirm scoring. Genotype data for each individual for the 10 polymorphic loci were analysed using the BIOSYS computer program (Swofford & Selander, 1981) and the Genetic Data Analysis (GDA) computer program version 1.0 (Lewis & Zaykin, 1997). Hierarchical *F* statistics (Wright, 1951; Crow & Aoki, 1984), genetic distances between populations and measures of genetic variability were calculated. Dendrograms based on genetic distance measures were constructed using UPGMA (Sneath & Sokal, 1973) and neighbour-joining methods.

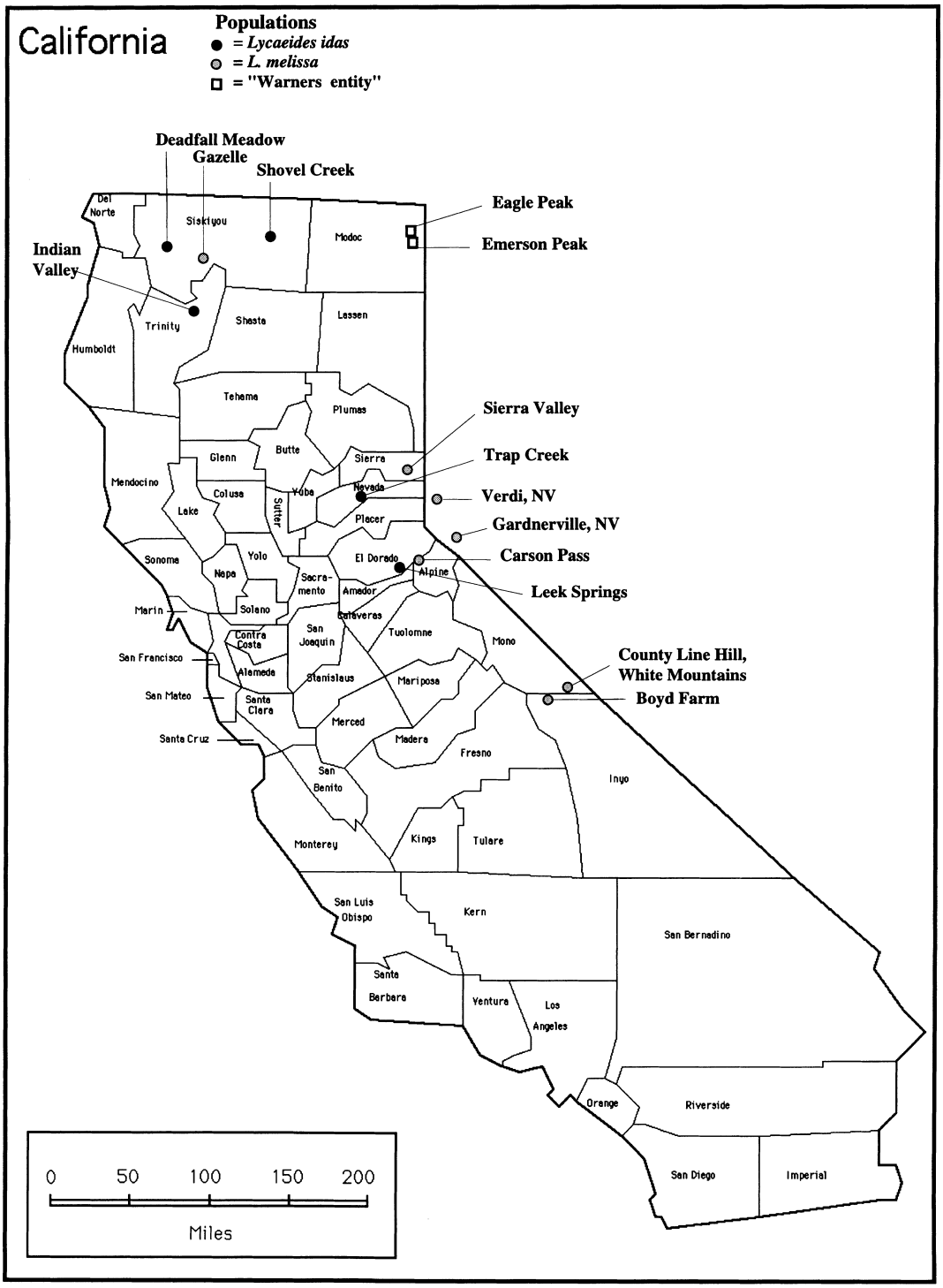


**Fig. 3** The locations of populations of *Lycaeides idas*, *L. melissa* and the Warners entity that were surveyed using allozyme electrophoresis.

### mtDNA methods

A 440-bp region of the mtDNA cytochrome oxidase subunit 1 region (COI) was sequenced for 38 specimens of *Lycaeides* plus several specimens of potential outgroup taxa. These specimens represent samples from 10 populations surveyed for allozyme variation, plus specimens from Nevada, Minnesota, Wisconsin and Michigan. Appendix C provides locality data. Samples used in the mtDNA sequence survey included specimens from several sources. Five specimens were previously processed for allozyme electrophoresis (see above) and the material from these homogenates was extracted. Twenty-eight fresh caught specimens were either kept alive until placed in –80 °C freezers at UC Davis or frozen in liquid nitrogen in the field and transported to –80 °C freezers. These include five specimens from Wisconsin donated by A. Wolf and B. Borth. Five others were pinned museum specimens provided by G. Balogh. Regardless of the origins of the samples, all underwent identical procedures for extraction, amplification and sequencing of mtDNA.

Wings and abdomens were removed in the same fashion as described in the allozyme electrophoresis methods (above) and the remaining body placed in an autoclaved 1.5-mL microcentrifuge tube. For those samples that were originally prepared for electrophoresis, an unmeasured portion of the electrophoresis homogenate was transferred to a new autoclaved 1.5-mL microcentrifuge tube. In Dr H. B. Shaffer's laboratory at UC Davis, chloroform-phenol extractions were performed following standard methods (Hillis *et al.*, 1996). Extracted DNA was rehydrated with 30 $\mu$L of $H_2O$. From these solutions, diluted solutions (1/100 or 1/200 dilutions of the original 30 $\mu$L) were made yielding a concentration of DNA of approximately 1–10 ng $\mu L^{-1}$. These solutions were used in PCR reactions (as described in Kocher *et al.*, 1989; Palumbi, 1996) employing the primers C1-J-1751 and C1-N-2191 (Simon *et al.*, 1994) yielding an approximately 440-bp PCR product which was then sequenced. Fluorescently labelled dideoxy terminators were used for single stranded sequencing reactions according to Applied Biosystems Inc. specifications. Labelled extension products were separated on a gel and analysed with an automated DNA sequencer (Applied Biosystems model 377) at the DNA sequencing facility, Division of Biological Sciences, University of California, Davis. From these sequences, 410 continuous nucleotides could be reliably read for all specimens. This portion of the CO1 region of the mtDNA genome corresponds approximately to positions 1781 through 2191 of the *Drosophila yakuba* reference sequence (Simon *et al.*, 1994). Parsimony analysis and phylogeny reconstructions based on these sequences were performed with the PAUP 3.1.1 program (Swofford, 1993); neighbour-joining using Kimura two-parameter corrected distances and Jukes-Cantor corrected distances was performed with the PHYLIP 3.5 program (Felsenstein, 1989). A nested analysis of variance (AMOVA; Excoffier *et al.*, 1992) was performed by partitioning the total sum of squares into components representing variation among individuals within populations, among populations within species and among species (the two Warner Mountains populations were omitted from this analysis) using the WINAMOVA program written by Dr L. Excoffier, University of Geneva.

## Results

### Genitalia

Of the 282 specimens of *L. idas* and *L. melissa*, 97.5% were correctly classifiable with the discriminant function analysis (Table 1) calculated from two of the five measurements (*F* and *U*) that accounted for 83% of the variation between the two species. All 96 specimens of *L. idas* and 179 of 186 specimens of *L. melissa* were

**Table 1** Results of discriminant analysis. (A) Results of multivariate analysis of variance with all five measurements (F, H, FH, U, E; Fig. 2). (B) Results of multivariate analysis of variance with two measurements (F and U; Fig. 2). (C,D) Species determinations (rows) are based on wing phenotype. Predicted species (columns) are classified by the discriminant function. In C, only specimens of *L. idas* and *L. melissa* were used to create the discriminant function. In D, the Warners entity was included as a third taxon.

| Source of variation | d.f. | Sum of squares | Mean square | *F* Value |
|---|---|---|---|---|
| A. All five measurements | | | | |
| Among species | 5 | 0.43 | 0.09 | 289.65 |
| Within species | 276 | 0.08 | 0.00 | |
| Total | 280 | 0.52 | | |
| B. Measurements F and U only | | | | |
| Among species | 2 | 0.36 | 0.18 | 667.94 |
| Within species | 279 | 0.08 | 0.00 | |
| Total | 280 | 0.44 | | |

| C | Predicted species | | |
|---|---|---|---|
| Species | *L. idas* | *L. melissa* | Totals |
| *L. idas* | 96 | 0 | 96 |
| *L. melissa* | 7 | 179 | 186 |
| Totals | 103 | 179 | 282 |
| 'Warners' | 27 | 2 | 29 |

| D | Predicted species | | | |
|---|---|---|---|---|
| Species | *L. idas* | *L. melissa* | 'Warners' | Totals |
| *L. idas* | 87 | 0 | 9 | 96 |
| *L. melissa* | 0 | 169 | 17 | 186 |
| 'Warners' | 1 | 0 | 28 | 29 |
| Totals | 88 | 169 | 54 | 311 |

identified correctly. The seven misclassified *melissa* came from two populations: 6 of 9 from Mt. Rose, Washoe County, NV, and 1 of 18 specimens from the White Mountains, Mono County, CA, were misclassified as *idas*. Means and standard deviations for each of the five measurements are given in Table 2. Beyond this quantitative assessment of morphology, we found that male genitalia of each species correctly fit Nabokov's (1949) descriptions: the form of the armature of *melissa* is more narrow and angular with the length of the falx and uncus being the dominant features. In contrast, *idas*, besides having a shorter falx and uncus, has a more rounded armature with less angularity. Figure 2 illustrates these qualitative differences. *Lycaeides idas* and *L. melissa*, as recognized by wing phenotypes, possess consistent and recognizable differences in male genital morphology.

The Warners entity's *idas*-like genitalia was confirmed: 27 of 29 specimens were classified as *idas* (Table 1C, Fig. 2). This is in contrast to their wing patterns which appear to possess the features of *melissa*. Though classified primarily as *idas*, Table 2 clearly shows that the Warners entity's means for all five genitalic measurements vary from the *idas* means and always in the direction of *melissa* (i.e. in a direction that is intermediate between *idas* and *melissa*). For example, the Warners entity's mean for falx length (*F* in Table 2) is 2.4 standard deviations above the *idas* mean, but 3.7 standard deviations below the *melissa* mean falx length.

Another way to visualize the intermediacy of the Warners entity is to examine the results of the discriminant analysis performed with the Warners entity treated as a third 'species' (Table 1D). An almost equal proportion of *L. idas* and *L. melissa* was misidentified as Warners entity in this analysis. Nine of 96 (9.4%) *L. idas* were misidentified, including individuals from the Indian Valley CA, Mt. Ashland, OR, and Marinette, WI, populations. Seventeen of 186 (9.1%) *L. melissa* were misidentified, including individuals from the Boyd Farm, CA, County Line Hill, CA, and Carson Pass, CA, populations and all nine individuals from Mt. Rose, NV. Indeed, the Mt. Rose population, with *melissa* wing phenotypes and intermediate genitalic measurements, appears to be very similar to the Warner Mountains populations.

**Table 2** Means (SD), in 1/100 mm units, by species for the five genitalic measurements: F = falx length, H = humerulus length, FH = falx to humerulus length, U = uncus length, E = elbow width, following Nabokov (1949).

| | *Lycaeides melissa* *N* = 186 | *Lycaeides idas* *N* = 96 | 'Warners Entity' *N* = 29 |
|---|---|---|---|
| F | 58.68 (4.8) | 40.16 (2.4) | 45.93 (2.0) |
| H | 38.19 (2.6) | 33.89 (2.2) | 34.07 (1.5) |
| FH | 37.08 (4.4) | 26.89 (3.4) | 30.07 (3.6) |
| U | 39.49 (3.0) | 28.66 (2.7) | 31.38 (3.4) |
| E | 7.39 (0.9) | 8.62 (0.9) | 8.0 (0.8) |

## Allozymes

Allele frequencies of the polymorphic loci and measures of population genetic variability are similar in all populations surveyed (Table 3). Genetic distances were very small between all populations, regardless of species. (These distances are pairwise comparisons of genetic relationships between populations based on similarities in allele frequencies for the 10 polymorphic loci surveyed electrophoretically.) These distances were employed to construct a phenogram using UPGMA (Sneath & Sokal, 1973) (Fig. 4) demonstrating that *L. idas* and *L. melissa* are indistinguishable. The dendrograms drawn using other genetic distance metrics and the neighbour-joining tree of Nei's (1978) genetic distances were not significantly different from Fig. 4 and are not presented.

The range of genetic distances (Nei's $D = 0.002$–$0.078$) falls within the range of distances observed for conspecific populations of other invertebrates (Thorpe, 1983) indicating low levels of differentiation between all of the populations surveyed, regardless of their specific identification. Indeed, these genetic distances are characteristic of the level of differentiation of subspecies in other butterflies (Shapiro & Geiger, 1986; Porter & Geiger, 1988; Porter & Mattoon, 1989). The two species cannot be distinguished using these allozyme data (Fig. 4).

## mtDNA

The 410-bp sequences of 38 *Lycaeides* specimens and outgroup taxa specimens were A-T rich, with A-T content ranging from 69 to 71%. This range is commonly observed in insect mtDNA sequences (Simon *et al.*, 1994). (The rest of these results will consider *Lycaeides* exclusively.) Twenty-eight (6.8%) of the 410 bp sequenced from mtDNA of *Lycaeides* specimens were found to be variable. Thirteen haplotypes were revealed in this analysis with the most common haplotype (A) present in 16 specimens (see appendix C). The uncorrected sequence divergence between sequences ranged from 0.0% to 3.9%. Variable sites were strongly biased toward transitions with an average transition frequency across all pairwise haplotype comparisons equal to 76%. Haplotype diversity ($h$) was 0.80 (Nei, 1978) and nucleotide diversity ($\pi$) for the entire data set was 0.0096 (equation 10.5 in Nei, 1978). Protein sequences were inferred using an mtDNA code from *Drosophila* with eight codons for serine. Three of the 136 amino acid residues varied across all *Lycaeides* specimens.

The distribution of mtDNA sequence variation failed to distinguish the two species. Figure 5 is a strict consensus cladogram of the mtDNA sequences using a model of evolution that weights transversions five times more heavily that transitions. Sequences from members of both *idas* and *melissa* are present in many of the clades, the exceptions being the distinct clade of alfalfa-feeding

**Table 3** Allele frequencies of the 10 polymorphic allozyme loci for all populations. Sample sizes are provided in the first row for every locus. Population abbreviations and locality and collection data may be found in Appendix B.

| Locus/alleles | | IV | DM | TC | LS | SC | CP | GV | SV | GZ | VE | BF | CH | EM | EA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK | | 20 | 20 | 20 | 20 | 10 | 20 | 20 | 20 | 20 | 16 | 18 | 20 | 9 | 9 |
| | A | 0.975 | 0.900 | 1.000 | 0.925 | 0.850 | 0.750 | 0.875 | 1.000 | 0.800 | 0.969 | 0.750 | 0.950 | 0.944 | 0.944 |
| | B | 0.025 | 0.075 | 0.000 | 0.075 | 0.150 | 0.200 | 0.100 | 0.000 | 0.000 | 0.031 | 0.250 | 0.025 | 0.000 | 0.056 |
| | C | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.200 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | D | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.026 | 0.000 |
| FBA | | 20 | 16 | 11 | 20 | 15 | 20 | 19 | 17 | 16 | 17 | 14 | 15 | 20 | 4 |
| | A | 0.950 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.938 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | B | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.062 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| GPI | | 19 | 20 | 20 | 20 | 14 | 19 | 17 | 14 | 17 | 19 | 10 | 15 | 20 | 9 |
| | A | 0.474 | 0.700 | 0.975 | 0.975 | 0.786 | 0.763 | 0.235 | 0.607 | 0.353 | 0.447 | 0.700 | 0.333 | 0.500 | 0.556 |
| | B | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.706 | 0.250 | 0.000 | 0.184 | 0.100 | 0.200 | 0.200 | 0.222 |
| | C | 0.421 | 0.300 | 0.025 | 0.000 | 0.214 | 0.026 | 0.029 | 0.107 | 0.441 | 0.105 | 0.100 | 0.033 | 0.300 | 0.111 |
| | D | 0.079 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.029 | 0.132 | 0.000 | 0.033 | 0.000 | 0.000 |
| | E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.067 | 0.000 | 0.000 |
| | F | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.184 | 0.000 | 0.036 | 0.176 | 0.132 | 0.100 | 0.333 | 0.000 | 0.111 |
| | G | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.029 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G3PDH | | 20 | 20 | 20 | 20 | 15 | 20 | 20 | 20 | 20 | 20 | 18 | 20 | 20 | 9 |
| | A | 0.950 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | B | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| HK-1 | | 20 | 19 | 20 | 20 | 15 | 20 | 20 | 20 | 20 | 17 | 18 | 20 | 17 | 9 |
| | A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.975 | 1.000 | 0.972 | 1.000 | 1.000 | 1.000 |
| | B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.028 | 0.000 | 0.000 | 0.000 |
| HK-2 | | 11 | 14 | 15 | 16 | 8 | 11 | 5 | 10 | 15 | 14 | 18 | 20 | 12 | 9 |
| | A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.967 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| IDH-1 | | 20 | 20 | 13 | 20 | 15 | 16 | 20 | 17 | 15 | 20 | 18 | 20 | 20 | 9 |
| | A | 0.950 | 1.000 | 0.923 | 0.950 | 1.000 | 0.969 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.950 | 1.000 |
| | B | 0.050 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | 0.000 |
| | C | 0.000 | 0.000 | 0.077 | 0.025 | 0.000 | 0.031 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| IDH-2 | | 19 | 20 | 13 | 20 | 15 | 16 | 20 | 17 | 15 | 20 | 18 | 20 | 20 | 9 |
| | A | 1.000 | 1.000 | 0.962 | 0.975 | 1.000 | 0.969 | 1.000 | 1.000 | 0.967 | 1.000 | 0.806 | 1.000 | 0.900 | 1.000 |
| | B | 0.000 | 0.000 | 0.038 | 0.025 | 0.000 | 0.031 | 0.000 | 0.000 | 0.033 | 0.000 | 0.194 | 0.000 | 0.100 | 0.000 |
| MDH | | 19 | 6 | 14 | 13 | 15 | 14 | 15 | 10 | 13 | 20 | 18 | 20 | 20 | 9 |
| | A | 0.974 | 1.000 | 1.000 | 1.000 | 0.967 | 0.929 | 0.967 | 0.950 | 1.000 | 0.825 | 0.944 | 0.975 | 0.925 | 0.944 |
| | B | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.071 | 0.000 | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | 0.000 | 0.056 |
| | C | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.056 | 0.000 | 0.000 | 0.000 |
| | D | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 |
| | E | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.000 | 0.000 | 0.050 | 0.000 | 0.050 | 0.000 | 0.025 | 0.075 | 0.000 |
| PGM | | 19 | 20 | 20 | 20 | 15 | 19 | 20 | 20 | 20 | 20 | 17 | 20 | 18 | 9 |
| | A | 0.895 | 0.800 | 0.950 | 0.850 | 0.967 | 0.895 | 0.500 | 0.600 | 0.775 | 0.675 | 0.706 | 0.750 | 0.806 | 0.889 |
| | B | 0.000 | 0.075 | 0.025 | 0.150 | 0.033 | 0.053 | 0.400 | 0.400 | 0.175 | 0.225 | 0.265 | 0.200 | 0.111 | 0.000 |
| | C | 0.079 | 0.125 | 0.025 | 0.000 | 0.000 | 0.000 | 0.075 | 0.000 | 0.050 | 0.075 | 0.029 | 0.050 | 0.083 | 0.111 |
| | D | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | E | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 |
| | F | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

*L. melissa* from Verdi and Gardnerville, NV which all have identical sequences (haplotype K) and the individuals (*L. idas*) from Deadfall Meadow, CA, which also have identical sequences (haplotype H). Furthermore, no clear geographical patterns are evident. Among the 16 individuals with haplotype A (the most common) are specimens of both species from Wisconsin, Minnesota, eastern Nevada, the Warner Mountains (Warners entity) in California, southern California and south-western Oregon. Cladograms drawn using other models of

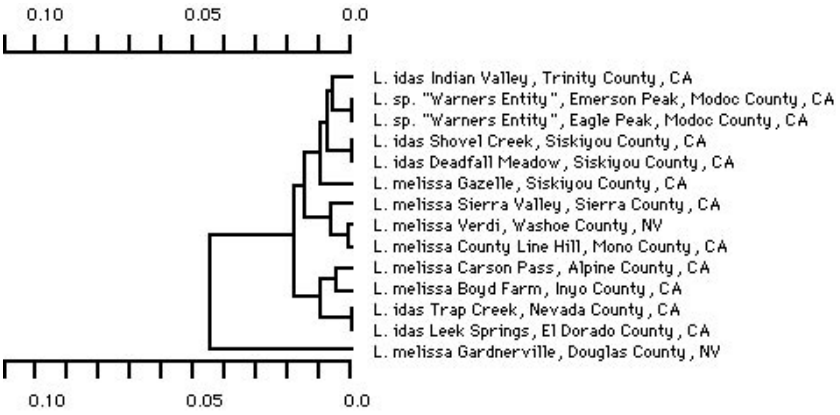


**Fig. 4** Nei's (1978) genetic distance phenogram calculated from allozyme frequency data from *L. idas* and *L. melissa* populations clustered using UPGMA (Sneath & Sokal, 1973). This dendrogram suggests that these species are closely related and cannot be distinguished electrophoretically.

evolution (i.e. other weightings) and the tree constructed using neighbour-joining of corrected sequence distances do not differ significantly from that constructed using parsimony.

The AMOVA analysis clearly illustrates the absence of differentiation among the species. The among-species component of variance is a very small fraction of the total variance and is not significantly different from zero ($P = 0.31$; Table 4B).

## Discussion

Results of the analysis of male genital morphology confirms the division of *L. idas* and *L. melissa* in contrast to the allozyme and mtDNA results. This division in genital morphology agrees with differences in wing pattern and habitat preference. The Warners entity's *idas*-like male genitalia was confirmed, though their genital morphology also suggests that the Warners entity may form a separate taxon, distinct from either *idas* or *melissa* (Table 2). Overall, the morphology-based discriminant analysis misidentified only 2.5% of the specimens (Table 1). However, this morphological differentiation is in sharp contrast to an almost complete lack of taxonomic and geographical structure in allozymes and mtDNA. In neither genetic data set were the two species distinguishable, to say nothing of the lack of subspecific differentiation. The genetic distances of the pairwise

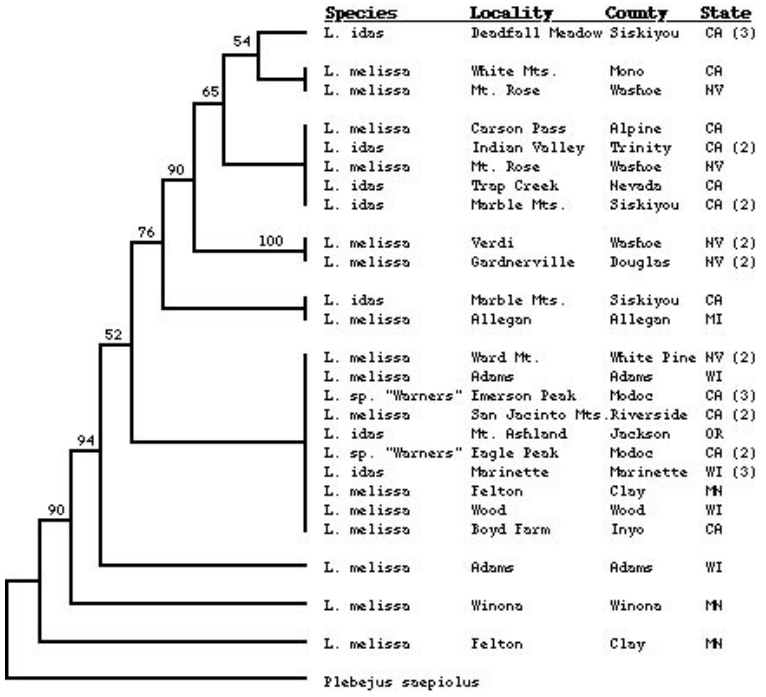


**Fig. 5** A strict consensus cladogram of *Lycaeides* mtDNA sequences using a model of evolution that weights transversions five times more heavily than transitions. Bootstrap values from 100 bootstrap replicates are above branches.

**Table 4** (A) Summary of hierarchical *F* statistic means at different levels calculated using the formulae given in Weir (1996). These values are calculated from allozyme allele frequencies over the 10 polymorphic loci surveyed in 14 populations of *L. idas* and *L. melissa* from California and western Nevada. (Appendix B provides locality data.) Confidence intervals were calculated from 1000 bootstrap replicates over all loci. (B) Nested analysis of molecular variance (AMOVA) for 33 individuals in 19 populations (Warner Mountains populations excluded) surveyed for mtDNA sequence variation. Populations are grouped into the two species: *Lycaeides idas* and *L. melissa*. d.f.: degrees of freedom, SSD: sums of squares, MSD: mean squared deviations, *P*-value: probability of obtaining a larger component of variance by chance under the null hypothesis that the variance component is zero (estimated from 1000 permutations).

| A. | Hierarchical level | Value | 95% CI |
|---|---|---|---|
| | $F_{IS}$ | 0.136 | 0.311–0.024 |
| | $F_{IT}$ | 0.238 | 0.376–0.093 |
| | $F_{ST}$ | 0.118 | 0.170–0.027 |

| B. Source of variation | d.f. | SSD | MSD | Variance component | % of total | *P*-value |
|---|---|---|---|---|---|---|
| Among species | 1 | 9.47 | 9.47 | 0.09 | 2.3 | 0.31 |
| Among populations/within species | 17 | 107.40 | 6.32 | 3.19 | 75.9 | <0.001 |
| Among individuals/within populations | 14 | 12.83 | 0.92 | 0.92 | 21.8 | <0.001 |

population comparisons of allozyme allele frequencies (Nei's $D = 0.002–0.078$) are very low and within the range of conspecific populations of other invertebrates including butterflies (Thorpe, 1983). Furthermore, mtDNA sequence data support the results from the allozyme analysis that these butterflies appear to be indistinguishable genetically despite the existence of considerable variation. The phylogeny reconstructed from the sequence data also illustrates the absence of geographical structure for variation in the CO1 gene. For example, haplotype A, the most common haplotype, occurs in 26 specimens whose populations are separated by huge geographical distances (>2000 km) and this is true of most of the other haplotypes that recur in the data. (Because many populations in the mtDNA data set are represented by a single individual, estimation of $M$ ($=Nm$) among populations is problematic. Consequently, a test for isolation by distance (Slatkin, 1993) is not presented.) The only clear differentiation in the mtDNA data is observed in the clade containing four sequences from individuals of *L. m. melissa* from Verdi and Gardnerville, Nevada. In short, despite clear differences in habitat preference, wing patterns (Nabokov, 1949; Ferris & Brown, 1981; Garth & Tilden, 1986; Scott, 1986; Tilden & Smith, 1986; Opler, 1992) and male genitalic morphology (Nabokov, 1949; demonstrated here), *L. idas* and *L. melissa* are indistinguishable using 10 polymorphic allozyme loci or 410-bp sequences of the mitochondrial CO1 gene.

It seems unlikely that selection would be operating on a variety of nuclear markers (allozyme loci) and mtDNA such that effectively all 10 allozyme loci and one mtDNA locus show the same lack of differentiation between populations of *idas* and *melissa*. Additionally, although environmental factors commonly affect wing patterns in butterflies (Shapiro, 1974, 1976) and have been shown to affect male genital morphology in one case (Shapiro, 1978), such phenomena are qualitatively different from what is seen in *Lycaeides*. It is difficult, though not impossible, to imagine that phenotypic plasticity is responsible for the differences between *idas* and *melissa* across a large portion of North America. We consider this unlikely, but careful rearing and common garden experiments are required to reject this hypothesis. We have reared butterflies from a few populations without observing any tendency to this sort of variation. Unfortunately, attempts at even intraspecific matings in our laboratory have so far been unsuccessful. Though we cannot rule out these possibilities, we feel that congruent selection and phenotypic plasticity are unlikely explanations for the lack of genetic differentiation.

The lack of congruence in the patterns of variation in morphology and genetic markers in these butterflies might be explained by two alternative, but not necessarily mutually exclusive, hypotheses: (1) considerable gene flow at neutral loci and/or reticulation between *idas* and *melissa*, perhaps due to the recency of speciation, or (2) insufficient lineage sorting, again possibly due to the recency of speciation. Each of these alternatives will be discussed in turn.

Hypothesis 1 posits that gene flow is occurring or has occurred recently. Arnold (1997, p. 29) notes that 'nonconcordance between the placement of taxa using genetic vs. morphological characters' in phylogenies may be strong evidence of reticulation. Though we did not construct a phylogeny based on morphological data, it is clear that *idas* and *melissa* are consistently recognizable, but that gap in morphological space is not paralleled by allozyme or mtDNA markers. If gene flow is occurring, it must be countered by selection maintaining morpological and ecological distinctions. Nabokov (1949, p. 540) speculated on the possibility of gene flow between the species by noting that *L. i. longinus* from north-western Wyoming, 'although taxonomically assigned to [*idas*],

includes transitions between the latter and *melissa*'. The populations in the Warner Mountains of north-eastern California, where individuals were classified as *idas* based on male genital morphology, but possess wing patterns similar to *melissa*, suggest the possibility of gene flow and hybridization. If gene flow is occurring (or has recently occurred) between the species we can estimate the level of migration between populations using the $F_{ST}$ statistic (Table 4A; Wright, 1951; Weir & Cockerham, 1984; Slatkin, 1987) calculated from allozyme data assuming an island model: $Nm = 1.9$ migrants/generation, theoretically enough gene flow to homogenize allele frequencies across populations, and, in this case, between species, if populations are currently at equilibrium (Slatkin, 1987). The AMOVA analysis (Table 4B) shows an analogous lack of differentiation among species.

Despite a great deal of attention being paid to these butterflies because of the conservation status of several subspecies (Arnold, 1993; Andow *et al*., 1994), no clear evidence of interbreeding or even immediate sympatry – anywhere – is known to us. Shepard (1964) notes that there is no evidence of immediate sympatry or interbreeding in the Pacific Northwest. There is also no clear evidence that the Warner Mountains populations are of hybrid origin, despite their phenotypic intermediacy. There are no rare alleles or private alleles specific to either species that could be used to detect hybrids. There are no immediately nearby populations of *idas* that could be acting as sources of dispersants, though the alfalfa-feeding agricultural *melissa* occurs in Surprise Valley just east of the Warner range. All of this suggests that if gene flow is responsible for the lack of geographical genetic structure, it does not appear to be contemporary gene flow, but rather it has occurred in the recent past. The fact that the Warners entity also occurs on Ball Mountain, Siskiyou County, CA, 225 km to the south-west, along with several other Great Basin relicts (Shapiro, 1991) also suggests that it has been differentiated for some time, whatever its mode of origin.

An alternative to the hypothesis of gene flow is the possibility that there is a lack of lineage sorting among these butterflies because speciation occurred recently (or is ongoing) (Niegel & Avise, 1986). This idea requires that the rates of morphological evolution be greater than the rates of molecular evolution. This situation is not unprecedented. Zink & Dittman (1993) concluded that subspecific morphological differentiation in Song Sparrows (*Melospiza melodia*) has occurred after a recent range expansion and at a greater rate than mtDNA evolution (see also Fry & Zink, 1998). Similarly in other birds, little or no phylogeographic structure was detected, despite morphological differentiation in Brown Headed Cowbirds (*Molothrus ater*) (Ball & Avise, 1992), Red-winged Blackbirds (*Agelaius phoeniceus*) (Ball *et al*., 1988), the common grackle (*Quiscalus quiscala*) (Zink *et al*., 1991) and Redpolls (*Carduelis* spp.) (Seutin *et al*., 1995). This pattern has also been found across species boundaries in King and Hermit crabs (Cunningham *et al*., 1992). In these cases, rates of morphological evolution appear to be greater than rates of molecular evolution.

For *idas* and *melissa*, the rates of evolution in wing patterns and male genital morphology appear to be substantially greater than the rates of allozyme and even mtDNA differentiation. Strong selection is required for this model, as it is for the alternative explanation involving significant gene flow. However, it is easier to imagine this selection operating quickly without the counteracting effects of gene flow (i.e. selection would need to be less intense without gene flow). There may be even more justification for accepting the hypothesis that recent selection played a significant role if wing patterns and male genital morphology are experiencing, or have experienced, sexual selection. Traits acted upon by sexual selection may experience more rapid evolution than other traits (Kosswig, 1947; West-Eberhard, 1983). It is also possible that the differences in male genital morphology are mirrored in female genital morphology or behaviour and represent a premating reproductive isolating barrier preventing gene flow (Shapiro & Porter, 1989; Porter & Shapiro, 1990).

We feel that the hypotheses of gene flow or an insufficiency of lineage sorting are viable hypotheses. It is important to point out that without breeding experiments to assess the amount and nature of reproductive isolation (i.e. are wing pattern, genital and habitat differences responsible for reproductive isolation as we believe they may be?), we cannot discount the possibility that significant gene flow or reticulation is responsible for the patterns, or accept the hypothesis that lineage sorting is currently underway. However, both of these possibilities strongly suggest that *idas* and *melissa* are very recently diverged and both hypotheses have interesting evolutionary implications regarding the relative roles of selection and drift in the speciation process.

A controversial question in speciation studies and theories has been whether reproductive isolating barriers are the products of natural (or sexual) selection on isolating traits, or whether they are the accidental byproducts of genetic drift in isolated populations (Mayr, 1942, 1963; Ehrlich & Raven, 1969; Slatkin, 1987; Coyne, 1992; Sperling, 1994). If we accept for the moment the hypothesis of insufficient time for lineage sorting, then we must conclude that habitat selection, wing patterns and male genital morphology have evolved much more rapidly than mtDNA or allozymes because these morphological and ecological traits appear widely repeated in space, but mtDNA and allozymic differentiation has not yet occurred. Speciation is not therefore complete, in the sense that reciprocal monophyly has not been achieved (Niegel & Avise, 1986; Slatkin, 1987). This implies the possibility that habitat preference, wing patterns and/or male genital morphology are important reproductive isolating traits actually responsible for speciation, and that geographical isolation and

subsequent differentiation by genetic drift played a relatively less important role in the origin of these butterfly species than did selection. This contradicts the expectation that species differences arise as byproducts of drift in isolation.

Butterfly speciation may be driven by a variety of mechanisms. Shapiro (1990) described a situation within the Pierid genus *Hypsochila* in which larval and pupal morphology and hostplant were strongly differentiated between two species whose male genitalia were so similar that the most recent reviser of the genus was unwilling to delimit species based on them. This situation does not comport with the usual models of genitalic differentiation at the species level, but does support speciation grounded in ecological differentiation.

If, on the other hand, gene flow is occurring or has recently occurred, then we must conclude that reproductive isolating barriers have not fully evolved. Coyne & Orr (1989) demonstrated for *Drosophila* species that levels of both pre- and post-zygotic isolation are correlated with Nei's *D* and thus also with time since divergence. Introgression should therefore be more common among recently diverged taxa. The hypothesis of gene flow between *L. idas* and *L. melissa* also suggests that speciation is a recent event and that selection (overcoming gene flow) is significantly more important than drift and isolation in the speciation process. A careful investigation of populations of *idas* and *melissa* in north-western Wyoming, where Nabokov (1949) suspected gene flow, may be illuminating. Both species occur there and this region is close to locations of considerable hybridization in the moths of the genus *Hyalophora* (Lepidoptera: Saturniidae) described by Collins (1997). Thus, there may be historical biogeographical factors that make this area a good place to test the hypothesis of gene flow between *idas* and *melissa*. These implications and hypotheses await validation from hybridization studies and mapping of the genes responsible for reproductive isolation.

*Lycaeides idas* and *L. melissa* are indistinguishable using allozyme and mtDNA markers and there appears to be very little correlation between species boundaries (observed as clear differences of wing colour patterns, habitat preferences and male genital morphology) and these genetic markers. Evaluation of alternative hypotheses indicates that the best explanations may be an insufficiency of time for lineage sorting and/or substantial recent gene flow, both indicating that differentiation of *idas* and *melissa* is relatively recent. In the geographical context of morphological/ecological divergence of *L. idas* and *L. melissa*, and the lack of congruent differentiation in nuclear and mitochondrial markers (and thus the seemingly greater rate of morphological/ecological evolution), these hypotheses imply a relatively more significant role for selection rather than isolation and drift in the recent *Lycaeides* speciation event in North America. The application of molecular mapping techniques in combination with hybridization experiments and the characterization of the genes controlling reproductive isolation are necessary to test this hypothesis.

## Acknowledgments

We would like to thank the following people for their help with various aspects of the project: J. Emmel and R. W. VanBuskirk for help with specimen collection; George Balogh, Amy Wolf, B. Borth, D. Underwood for kindly donating specimens for genitalic and/or genetic analysis; and A. H. Porter for discussions and demonstration of allozyme techniques. We are especially grateful to the following past and present Shaffer lab members for technical help and use of their equipment: Dr B. Shaffer, Dr R. Voss, Dr M. McKnight, and S. Rollman. The drawings are by L. Ellis. E. Sotka and M. Rejmánek provided assistance with discriminant statistics. Two anonymous reviewers provided helpful comments. This work was supported in part by a Graduate Research Award from the Center for Population Biology, University of California, Davis, CA, to C.C.N., a Jastro Shields Research Scholarship from the University of California, Davis, CA, to C.C.N., and by a Research Fellowship from the White Mountain Research Station to C.C.N.

## References

Andow, R., Baker, J. & Lane, C.P., eds. 1994. *Karner Blue Butterfly: Symbol of a Vanishing Landscape. Misc. Publ. 84–1994*. Minn. Agric. Exp. Stat., University of Minnesota, St. Paul.

Arnold, R.A. 1993. The Lotis Blue, *Lycaeides Idas Lotis* (Lintner). In: *Conservation Biology of the Lycaenidae (Butterflies)* (T. R. New, ed.), Occasional Paper of the IUCN Species Survival Commission no. 8.

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

Ball, R.M., James, F.C., Freeman, S., Bermingham , E. & Avise, J.C. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. *Proc. Nat. Acad. Sci. USA* **85**: 1558–1562.

Ball, R.M. Jr & Avise, J.C. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**: 626–636.

Ballmer, G.R. & Pratt, G.F. 1988. A survey of last instar larvae of the Lycaenidae (Lepidoptera) of California. *J. Res. Lepidoptera* **27**: 1–81.

Collins, M.M. 1997. Hybridization and speciation in *Hyalophora* (Insecta: Lepidoptera: Saturniidae): a reappraisal of W. R. Sweadner's classic study of a hybrid zone. *Ann. Carnegie Museum* **66**: 411–455.

Coyne, J.A. 1992. Genetics and speciation. *Nature* **355**: 511–515.

Coyne, J.A. & Orr, H.A. 1989. Patterns of Speciation in *Drosophila*. *Evolution* **43**: 362–381.

Crow, J.F. & Aoki, K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of subdivision. *Proc. Natl. Acad. Sci., USA* **81**: 6073–6077.

Cunningham, C.W., Blackstone, N.W. & Buss, L.W. 1992. Evolution of King Crabs from Hermit Crab ancestors. *Nature* **355**: 539–542.

Ehrlich, P.R. & Raven, P.H. 1969. Differentiation of populations. *Science* **165**: 1228–1232.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Felsenstein, J. 1989. PHYLIP – phylogeny inference package. *Cladistics* **5**: 164–166.

Ferris, C.D. 1989. (Suppl.)to: a catalogue/checklist of the butterflies of America north of Mexico. *The Lepidopterists' Society, Memoir no. 3*.

Ferris, C.D. & Brown, F.M., eds. 1981. *Butterflies of the Rocky Mountain States*. University of Oklahoma Press, Norman.

Fry, A.J. & Zink, R.M. 1998. Geographic analysis of nucleotide diversity and song sparrow (Aves: Emberizidae) population history. *Mol. Ecol.* **7**: 1303–1313.

Garth, J.S. & Tilden, J.W. 1986. *California Butterflies*. University of California Press, Berkeley.

Higgins, L.G. 1985. The correct name for what has been called *Lycaeides argyrognomon* in North America. *J. Lep. Soc.* **39**: 145–146.

Hillis, D.M., Mable, B.K., Larson, A., Davis, S.K. & Zimmer, E.A. 1996. Nucleic acids IV: sequencing and cloning. In: *Molecular Systematics* (D. M. Hillis, C. Moritz & B. K. Mable, eds). Sinauer, Sunderland, MA.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X. & Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196–6200.

Kosswig, C. 1947. Selective mating as a factor for speciation in Cichlid fish of east African lakes. *Nature* **159**: 4044.

Lane, C.P. 1994. Habitat preferences of the Karner Blue Butterfly in Minnesota. In: *Karner Blue Butterfly: Symbol of a Vanishing Landscape. Misc. Publ. 84–1994* (R. Andow, J. Baker & C. P. Lane, eds). Minn. Agric. Exp. Stat., University of Minnesota, St. Paul.

Lane, C.P. & Weller, C.P. 1994. A review of *Lycaeides* Hubner and Karner Blue Butterfly taxonomy. In: *Karner Blue Butterfly: Symbol of a Vanishing Landscape. Misc. Publ. 84–1994* (R. Andow, J. Baker & C. P. Lane, eds). Minn. Agric. Exp. Stat., University of Minnesota, St. Paul.

Lewis, P.O. & Zaykin, D. 1997. Genetic Data Analysis: computer program for the analysis of allelic data, Version 1.0. Free program distributed by the authors over the internet from the GDA homepage at http://chee.unm.edu/gda/.

Ludwig, J.A. & Reynolds, J.F. 1988. *Statistical Ecology*. John Wiley and Sons, New York.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

Miller, L.D. & Brown, F.M. 1981. A catalogue/checklist of the butterflies of America north of Mexico. The Lepidopterists' Society, Memoir no. 2.

Nabokov, V. 1949. The nearctic members of *Lycaeides* Hübner (Lycaenidae, Lepidoptera). *Bull. Mus. Comp. Zoo*. **101**: 479–541.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

Niegel, J.E. & Avise, J.C. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory* (E. Nevo & S. Karlin, eds). Academic Press, New York.

Opler, P. 1992. *A Field Guide to Eastern Butterflies*. Houghton Mifflin Co., Boston.

Palumbi, S. 1996. Nucleic acids II: the polymerase chain reaction. In: *Molecular Systematics* (D. M. Hillis, C. Moritz & B. K. Mable, eds). Sinauer, Sunderland, MA.

Porter, A.H. & Geiger, H. 1988. Genetic and phenotypic population structure of the *Coenonympha tullia* complex (Lepidoptera: Nymphalidae: Satyrinae) in California: no evidence for species boundaries. *Can. J. Zool.* **66**: 2751–2765.

Porter, A.H. & Mattoon, S.O. 1989. A new subspecies of *Coenonympha tullia* (Müller) (Nymphalidae: Satyrinae) confined to the coastal dunes of northern California. *J. Lep. Soc.* **43**: 229–238.

Porter, A.H. & Shapiro, A.M. 1990. Genitalia and arthropod taxonomy: lack of mechanical isolation in a butterfly hybrid zone (Lepidoptera: Pieridae). *Ann. Ent. Soc. Amer.* **82**: 107–114.

Rejmánek, M. & Richardson, D.M. 1996. What attributes make some plants species more invasive? *Ecology* **77**: 1655–1661.

Scott, J.A. 1986. *The Butterflies of North America, a Natural History and Fieldguide*. Stanford University Press, Stanford, California.

Seutin, G., Ratcliffe, L.M. & Boag, P.T. 1995. Mitochondrial DNA homogeneity in the phenotypically diverse Redpoll Finch complex (Aves: Carduelinae: *Carduelis flammea-hornemanni*). *Evolution* **49**: 962–973.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C. & Whitt, G.S. 1990. Gene nomenclature for protein-coding loci in fish. *Trans. Amer. Fish. Soc.* **119**: 2–15.

Shapiro, A.M. 1974. Natural and laboratory occurrence of 'elmyi' phenotypes *in Cynthia cardui* (Nymphalidae). *J. Res. Lep.* **13**: 57–62.

Shapiro, A.M. 1976. Photoperiod and temperature in phenotype determination of pacific slope pierini: biosystematic implications. *J. Res. Lep.* **16**: 193–200.

Shapiro, A.M. 1978. The assumption of adaptivity in genital morphology. *J. Res. Lep.* **17**: 68–72.

Shapiro, A.M. 1990. The life histories and behavior of the Patagonian-Fuegian white butterflies *Hypsochila macrodice* and *H. galactodice* (Lepidoptera: Pieridae). *J. New York Entomol. Soc.* **98**: 461–473.

Shapiro, A.M. 1991. Ball Mountain revisited: anomalous species richness of a montane barrier zone. *J. Res. Lep.* **29**: 143–156.

Shapiro, A.M. 1995. From the mountains to the prairies to the ocean white with foam: *Papilio zelicaon* makes itself at home. In: *Genecology and Ecogeographic Races* (A. R. Kruckeberg, R. B. Walker & A. E. Leviton, eds). Pacific Division AAAS, San Francisco, California.

Shapiro, A.M. & Geiger, H. 1986. Electrophoretic confirmation of the species status of *Pontia protodice* and *P. occidentalis* (Pieridae). *J. Res. Lep.* **25**: 39–47.

Shapiro, A.M. & Porter, A.H. 1989. The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Ann. Rev. Entomol.* **34**: 231–245.

Shepard, J.H. 1964. The genus *Lycaeides* in the Pacific Northwest. *J. Res. Lepidoptera* **3**: 25–36.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Ent. Soc. Amer.* **87**: 651–701.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* **236**: 787–792.

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

Sneath, P.H.A. & Sokal, R.R. 1973. *Numerical Taxonomy*. W. B. Freeman, San Francisco.

Snedecor, G.W. & Cochran, W.G. 1967. *Statistical Methods*, 6th edn. Iowa State University Press, Ames, Iowa.

Sperling, F.A.H. 1994. Sex-linked genes and species differences in Lepidoptera. *Can. Entomol.* **126**: 807–818.

Stanford, R.E. & Opler, P.A. 1996. *Atlas of Western USA Butterflies, Including Adjacent Parts of Canada and Mexico*. Denver and Fort Collins, Colorado.

Swofford, D.L. 1993. PAUP: Phylogenetic analysis using parsimony, Version 3.1.1 computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D.L. & Selander, R.B. 1981. A computer program for the analysis of allelic variation in genetics. *J. Heredity* **72**: 281–283.

Thorpe, J.P. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: *Protein Polymorphism: Adaptive and Taxonomic Significance* (G. S. Oxford & D. Rollinson, eds), Systematics Association, Vol. no. 24. Academic Press, London.

Tilden, J.W. & Smith, A.C. 1986. *A Field Guide to Western Butterflies*. Houghton Mifflin Co., Boston.

US Fish and Wildlife Service. 1992. Department of the Interior. Endangered and Threatened Wildlife and Plants. Determination of endangered status for the Karner blue butterfly. Final rule. *Federal Register December 14, 1992*, **57** (240): 59236–44.

Weir, B.S. 1996. *Genetic Data Analysis*. Sinauer Associates, Sunderland Massachusetts.

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Quart. Rev. Biol.* **58**: 155–183.

Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* **15**: 323–354.

Zink, R.M. & Dittman, D.L. 1993. Gene flow, refugia, and evolution of geographic variation in the Song Sparrow (Melospiza melodia). *Evolution* **47** (3): 717–729.

Zink, R.M., Rootes, W.L. & Dittman, D.L. 1991. Mitochondrial DNA variation, population structure, and evolution of the Common Grackle (*Quiscalus quiscula*). *Condor* **93**: 318–329.

*Received 20 January 1999; accepted 24 February 1999*

**Appendix A:** locality and collection data for Male Genitalic Morphology Specimens.

| Population locality† | *n* | Collection date | Collector | County, State |
|---|---|---|---|---|
| *Lycaeides idas* | 96 | | | |
| Deadfall Meadow[a] | 9 | vii.22.90 | AMS | Siskiyou, CA |
| Indian Valley[b] | 15 | vii.21.90 | AMS | Trinity, CA |
| Leek Springs[c] | 20 | vii.26.90 | AMS | El Dorado, CA |
| Shovel Creek[c] | 9 | vii.14.91 | CCN | Siskiyou, CA |
| Trap Creek[c] | 9 | vii.27.90 | AMS | Nevada, CA |
| Nabensa[d] | 2 | vii.25.91 | *D*. Underwood | AK |
| Tok[d] | 2 | vii.18.91 | *D*. Underwood | AK |
| Mt. Ashland[a] | 15 | vii.12.96 (8) | CCN | Jackson, OR |
| | | vii.18.97 (2) | CCN | |
| | | vii.20.97 (5) | R. Van Buskirk | |
| Marrinette[e] | 4 | vii.17.94 | A. Wolf | Marinette, WI |
| Marble Mt.s[a] | 2 | ix.13.95 | CCN | Siskiyou, CA |
| Yuba Gap[c] | 9 | vii.11.95 | CCN | Nevada, CA |
| *Lycaeides melissa* 186 | | | | |
| Boyd Farm[f] | 12 | viii.24.92 | CCN, C. Swingley | Inyo, CA |
| County Line Hill/ White Mt.s[g] | 19 | vii.8.92 | CCN | Mono, CA |
| Carson Pass[g] | 18 | vii.26.90 | AMS, CCN | Alpine, CA |
| Gardnerville[f] | 14 | ix.12.90 | AMS, CCN | Douglas, NV |
| Gazelle[f] | 15 | viii.15.90 | AMS | Siskiyou, CA |
| Sierra Valley[f] | 13 | vii.11.90 | AMS | Sierra, CA |
| Verdi[f] | 10 | ix.12.91 | AMS, CCN | Washoe, NV |
| Ward Mt.[f] | 6 | vii.8.94 | CCN | White Pine, NV |
| Adams[h] | 12 | vi.3.78 | B. Borth | WI |
| Necadah[h] | 2 | viii.9.85 | B. Borth | WI |
| Allegan Co.[i] | 6 | vi.3.80 | G. Balogh | Allegan, MI |
| Wood Co.[i] | 6 | vi.13.80 (3) | G. Baolgh | Wood, WI |
| | | v.29.80 (3) | | |
| Burnette Co.[i] | 6 | v.28.80 | G. Balogh | Burnette, WI |
| Felton[h] | 6 | vi.9.80 (5) | G. Balogh | Clay, MN |
| | | vi.10.80 (1) | | |
| Winona, Co.[i] | 2 | vii.28.81 | G. Balogh | Winona, MN |
| Mt. Rose[g] | 9 | vii.22.93 (4) | CCN | Washoe, NV |
| | | vii.24.93 (5) | | |
| San Jacinto Mt.s[k] | 18 | vi.11.93 | J. Emmel | Riverside, CA |
| Jeff Davis Peak[g] | 12 | viii.13.95 | CCN | Alpine, CA |
| 'Warners Entity' | 29 | | | |
| Emerson Peak[j] | 20 | viii.11.91 | D. Olson, L. Farley | Modoc, CA |
| Eagle Peak[j] | 9 | vii.14.92 (8) | CCN | Modoc, CA |

†Superscripts indicate subspecies found at each locality: (a) *ricei*, (b) unnamed, (c) *anna*, (d) *alaskensis*, (e) *nabokovi*, (f) agriculutural *melissa*, (g) alpine *melissa*, (*h*) *melissa*, (i) *samuelis*, (j)'Warners entity', (k) *inyoensis*.

**Appendix B:** locality and collection data for allozyme electrophoresis samples. Population abbreviations are listed before each locality name.

| Abbrev. | Population locality† | *n* | Collection date | Collector | County, State |
|---|---|---|---|---|---|
| *Lycaeides idas* | | 95 | | | |
| IV | Indian Valley[b] | 20 | vii.21.90 | AMS | Trinity, CA |
| DM | Deadfall Meadow[a] | 20 | vii.22.90 | AMS | Siskiyou, CA |
| TC | Trap Creek[c] | 20 | vii.27.90 | AMS | Nevada, CA |
| LS | Leek Springs[c] | 20 | vii.26.90 | AMS | El Dorado, CA |
| SC | Shovel Creek[c] | 15 | vii.14.91 | CCN | Siskiyou, CA |
| *Lycaeides melissa* | | 138 | | | |
| CP | Carson Pass[g] | 20 | vii.26.90 | AMS | Alpine, CA |
| GV | Gardnerville[f] | 20 | ix.12.90 | AMS | Douglas, CA |
| SV | Sierra Valley[f] | 20 | vii.11.90 | AMS | Sierra, CA |
| GZ | Gazelle[f] | 20 | viii.15.90 | AMS | Siskiyou, CA |
| VE | Verdi[f] | 20 | ix.12.91 | AMS, CCN | Washoe, NV |
| BF | Boyd Farm[f] | 18 | viii.24.92 | CCN, C. Swingley | Inyo, CA |
| CH | County Line Hill/ White Mts.[g] | 20 | vii.8.92 | CCN | Mono, CA |
| 'Warners Entity' | | 29 | | | |
| EM | Emerson Peak[j] | 20 | viii.11.91 | *D*. Olson and L. Farley | Modoc, CA |
| EP | Eagle Peak[j] | 9 | vii.14.92 | CCN | Modoc, CA |

†Superscripts indicate subspecies found at each locality: (a) *ricei*, (b) unnamed, (c) *anna*, (d) *alaskensis*, (e) *nabokovi*, (f) agricultural *melissa*, (g) alpine *melissa*, (*h*) *melissa*, (i) *samuelis*, (j)'Warners entity'.

**Appendix C:** locality and collection data for mtDNA samples.

| Locality, County, State† | *n* | Collection date | Collector | Source‡ | Haplotype |
|---|---|---|---|---|---|
| *Lycaeides idas* | 13 | | | | |
| Deadfall Meadow, Siskiyou Co., CA[a] | 3 | vii.22.90 | AMS | F | H(3) |
| Indian valley, Trinity Co., CA[b] | 2 | vii.21.90 | AMS | H, F | I, J |
| Trap Creek, Nevada Co., CA[c] | 1 | vii.27.90 | AMS | F | I |
| Marble Mt.s, Siskiyou Co., CA[a] | 3 | ix.13.95 | CCN | F | E(1), I(2) |
| Mt. Ashland, Jackson Co., OR[a] | 1 | vii.12.96 | CCN | F | A |
| Marinette, Marinette Co., WI[e] | 3 | vii.17.94 | A. Wolf | F | A |
| *Lycaeides melissa* | 20 | | | | |
| Boyd Farm, Inyo Co., CA[f] | 1 | viii.24.92 | CCN | H | A |
| Carson Pass, Alpine Co., CA[g] | 1 | vii.26.90 | AMS | H | F |
| County Line Hill/White Mt.s Mono Co., CA[g] | 1 | vii.8.92 | CCN | F | G |
| Mt. Rose, Washoe Co., NV[g] | 2 | vii.24.93 | CCN | F | F, G |
| Verdi, Washoe Co., NV[f] | 2 | ix.12.91 | AMS, CCN | F | K |
| Gardnerville, Douglas Co., NV[f] | 2 | ix.12.90 | CCN | F | K |
| Allegan, Allegan Co., MI[i] | 1 | vi.3.80 | G. Balogh | P | D |
| Ward Mt., White Pine Co., NV[f] | 2 | vii.8.94 | CCN | F | A |
| Felton, Clay Co., MN[h] | 2 | vi.9.80 | G. Balogh | P | B, M |
| Adams, Adams Co., WI[h] | 2 | vi.3.78 | B. Borth | F | A, C |
| San Jacinto Mt.s, Riverside Co., CA[k] | 2 | vii.11.93 | J. Emmel | F | A |
| Wood Co., Wood Co., WI[i] | 1 | v.29.80 | G. Balogh | P | A |
| Winona Co., Winona Co., MN[i] | 1 | vii.28.81 | G. Balogh | P | L |
| 'Warners Entity' | 5 | | | | |
| Emerson Peak, Modoc Co., CA[j] | 3 | viii.11.91 | D. Olson, L. Farley | F | A |
| Eagle Peak, Modoc Co., CA[j] | 2 | vii.14.92 | CCN | H | A |

†Superscripts indicate subspecies found at each locality: (a) *ricei*, (b) unnamed, (c) *anna*, (d) *alaskensis*, (e) *nabokovi*, (f) agricultural *melissa*, (g) alpine *melissa*, (h) *melissa*, (i) *samuelis*, (j) 'Warners entity', (k) *inyoensis*.
‡F = extraction from live caught and frozen specimen, H = extraction from electrophoresis homogenate, P = extracted from pinned specimen.