

Patterns of Genitalic Morphology Around Suture Zones in North American *Lycaeides* (Lepidoptera: Lycaenidae): Implications for Taxonomy and Historical Biogeography

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ABSTRACT Within the North American *Lycaeides* (Hübner) fauna, there are at least three major lineages that exhibit extensive morphological and ecological variation, especially at suture zones where these lineages meet. We examined male genitalic morphology in *Lycaeides* populations spanning much of North America to evaluate the current taxonomy and to address questions about the patterns of morphological variation at suture zones and potential evolutionary processes responsible for the patterns. Our genitalic measurements were based on those of V. Nabokov who revised North American *Lycaeides* taxonomy in the 1940s. Canonical discriminant analysis validated Nabokov's original species designations, but it did not support many of his subspecific designations. Populations at a suture zone in the Great Lakes region are similar to populations on the east side of this zone. Populations at a western suture zone in the Sierra Nevada and adjacent ranges exhibit intermediate morphology between lineages on either side of this suture zone. We tested the hypothesis that contemporary gene flow contributes to the patterns of morphology in suture zones by testing for the increased variance in quantitative traits that is expected in a hybrid swarm. Based on a comparison of variances from populations within and outside of these suture zones, there is no evidence of current hybridization between lineages, with the sole exception of the population sample from the White Mountains of eastern California. The intermediate morphology and the general absence of increased variance within western suture zone populations imply that hybridization may have been important in the evolution of North American *Lycaeides*.

KEY WORDS Remington suture zones, hybridization, *Lycaeides*, morphology, Nabokov

Lycaeides (Lepidoptera: Lycaenidae) is a Holarctic genus of blue butterflies that includes a species complex in North America. Blue butterflies (blues) (Polyomatini) are small butterflies with characteristic structural blue coloration on the dorsal wing surfaces of males and include ≈32 species in North America (Scott 1986, Opler 1992). Nabokov (1943, 1949) revised the classification of North American members of *Lycaeides* using qualitative wing color pattern characters and quantitative characters of male genitalic morphology. He recognized two species: the Holarctic *Lycaeides argyrognomon* (Bersträsser) and the Nearctic *L. melissa* (Edwards). The Palearctic species *L. argyrognomon* was separated on genitalic grounds from *L. idas* (L.) by Higgins (1985), but *L. idas* in North America is often referred to as *L. argyrognomon* in the older Nearctic literature. The ranges of *L. idas* and *L. melissa* broadly overlap in western North America (Fig. 1A), with local populations occurring in sym-

patry or parapatry (Nabokov 1949; Layberry et al. 1998; Guppy and Shepard 2001; C.C.N., unpublished data). Nabokov also identified 15 subspecies and several other subspecies have been named subsequently; however, taxonomists disagree on the validity of subspecific designations (e.g., Scott 1986, Opler 1992, Guppy and Shepard 2001). This polytypic group of butterflies exhibits extensive local morphological (Nabokov 1943, 1949; Forister et al. 2006), ecological (Nice et al. 2002), and behavioral (Fordyce et al. 2002, Fordyce and Nice 2003) variation and represents an exceptional opportunity to examine geographic patterns of diversity. Here, we examine geographic variation in male genitalic morphology in the context of defining taxonomic boundaries, and we test hypotheses about the mechanisms responsible for generating this variation.

Nabokov (1949) recognized that boundaries between *Lycaeides* species were not discrete. There are several geographic locations in which *Lycaeides* populations have discordant wing pattern and genitalic characters; however, such claims have not been addressed with statistical rigor. For example, Nabokov (1949) hypothesized that *L. i. longinus* populations in the Teton range of northwestern Wyoming were of

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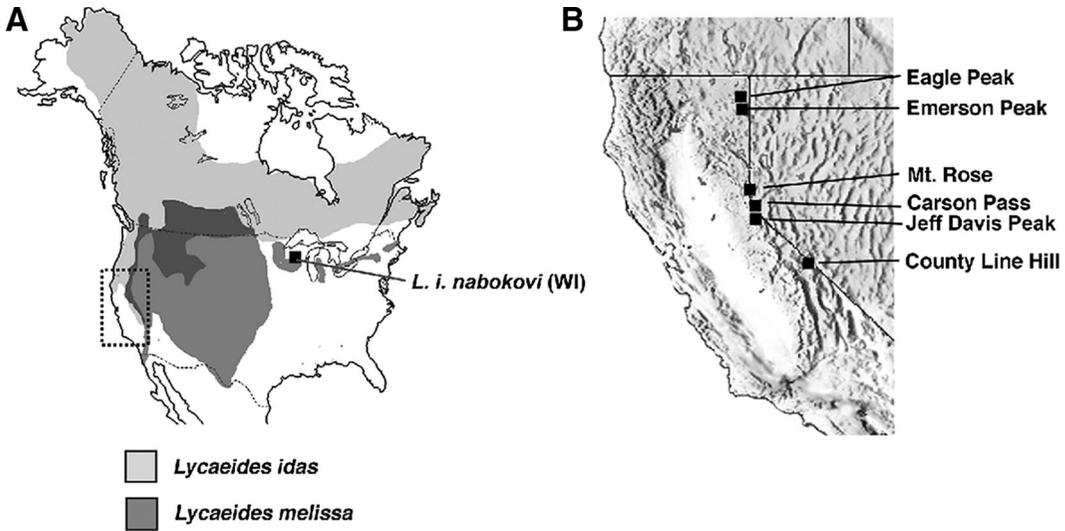


Fig. 1. (A) Locality map with ranges of North American *Lycaeides* species. The eastern portion of *L. melissa*'s range is the approximate historical range for *L. m. samuelis*. (B) Locations of alpine populations in suture zone VI, including three in the Sierra Nevada (Mt. Rose, Carson Pass, Jeff Davis Peak), one in the White Mountains (County Line Hill), and two in the Warner Mountains (Eagle Peak and Emerson Peak).

hybrid origin, a claim based on incongruence between wing patterns and male genitalic morphology. Additionally, the presumed extinct *L. i. lotis* (Arnold 1993) had genitalic morphology and habitat (bogs along the northern California coast) typical of the taxon *L. idas*, whereas its wing patterns were much more typical of *L. melissa*. Alpine populations in the Warner Mountains of northeastern California have variable wing patterns resembling *L. melissa* with male genitalic morphology closer to *L. idas* (C.C.N., unpublished data), whereas Sierra Nevada alpine populations have genitalic morphology intermediate to *L. idas* and *L. melissa* with *L. melissa* wing patterns (Nice et al. 2005). Another population in the White Mountains of central-eastern California exhibits morphological incongruities similar to Sierra Nevada populations (Nice et al. 2005). In Wisconsin, the state-endangered *L. i. nabokovi* also has intermediate genitalic morphology despite a *L. idas*-like wing pattern (Nice et al. 2005).

Phylogeographic investigations based on mitochondrial DNA (mtDNA) sequence variation, single copy nuclear DNA sequence variation, allozymes, microsatellites, and amplified fragment length polymorphism (AFLP) markers have indicated that there are at least three well-supported major lineages of *Lycaeides* in North America, representing a minimum of three Pleistocene refuges from which range expansion has occurred (Nice and Shapiro 1999; Nice et al. 2005; Gompert et al. 2006a,b). These three lineages roughly correspond to *L. idas*, *L. melissa*, and the endangered *L. m. samuelis*. This is consistent with previous suggestions that *L. m. samuelis* might be considered a unique entity or "good" species (Nabokov 1989, Cech and Tudor 2005, Gompert et al. 2006b). At the phylogeographic boundaries between lineages of North American *Lycaeides*, there is often incongruence be-

tween morphological characters and patterns of molecular genetic variation indicative of gene exchange where lineages meet (Nice et al. 2005; Gompert et al. 2006a,b). These areas correspond to two of the suture zones of Remington (1968) (Nice et al. 2005). Remington (1968) defined suture zones as areas of secondary contact and gene flow via hybridization between formerly isolated lineages. For *Lycaeides*, Pleistocene glaciations seem to have fostered divergence during glacial maxima, whereas post-Pleistocene range expansions created suture zones (Nice et al. 2005). Two suture zones in particular exhibit incongruence between patterns of mtDNA variation and ecological and morphological characters. *Lycaeides* populations in the alpine of the Sierra Nevada possess mtDNA haplotypes closely related to *L. idas* haplotypes, but they have wing patterns similar to *L. melissa* and recently have been shown to be a species of hybrid origin (Nice et al. 2005, Gompert et al. 2006a). This region corresponds to Remington's Pacific-Rocky Mountain suture zone VI. This area also was identified by Austin and Murphy (1987) as a "center of differentiation" where the edges of butterfly species ranges coincide along a biogeographical boundary, and by Swenson and Howard (2004, 2005) as a "hotspot of hybrid zone clustering." Based on mtDNA (Nice et al. 2005), another boundary between lineages within the *Lycaeides* species complex occurs roughly near Lake Michigan in Wisconsin and corresponds to Remington's Northeastern-Central suture zone I. In *Lycaeides*, this phylogeographical boundary separates eastern and western *L. m. samuelis* populations. Using data from mtDNA sequences and 143 polymorphic AFLP markers, Gompert et al. (2006b) showed that Wisconsin (western) populations of *L. m. samuelis* share mitochondrial haplotypes with *L. m.*

Table 1. Population data

Nominal taxonomic designation (N)	Pop	No. males measured	Nominal taxonomic designation (N)	Pop	No. males measured
<i>L. i. alaskensis</i> (18)	Healy, AK	3	<i>L. m. annetta</i> (15)	Alta, UT	15
	Walker River, AK	1		Big Pine, CA	26
	Deadhorse, AK	1	<i>L. m. alpine</i> (94)	Garner Valley, CA	18
	Nebesna, AK	3		San Diego Mts., CA	16
	Tok, AK	1		Mt. Rose, NV	24
	Dawson, YT	2		Carson Pass, CA	36
	Whitehorse, YT	4		The Nipple, CA	3
	Trevor Creek, AK	1		Jeff Davis Peak, CA	12
<i>L. i. anna</i> (98)	Koyukuk River, AK	2	County Line Hill, CA	19	
	Shovel Creek, CA	9	<i>L. m. samuelis</i> (232) (eastern) (61)	Concord, NH	5
	Trap Creek, CA	23		Saratoga, NY	25
	Yuba Gap, CA	25		Pine Bush/Albany, NY	2
Donner Pass, CA	20	Allegan, MI		6	
<i>L. i. azureus</i> (15)	Leek Springs, CA	21	(western) (171)	Indiana Dunes, IN	23
<i>L. i. nabokovi</i> (26)	Indian Valley, CA	15		Winona, MN	2
	Waubee Lake, WI	22		Fish Lake, WI	22
<i>L. i. ricei</i> (71)	Marinette, WI	4		Eau Claire, WI	22
	Mt. Ashland, OR	32		Black River, WI	18
	Marble Mts., CA	15		Sandhill, WI	21
	Cave Lake, CA	15		Welch/Hartman, WI	10
<i>L. m. melissa</i> (185)	Deadfall, CA	9		Fort McCoy, WI	25
	Range, MN	5		Necedah, WI	27
	Clay Co., MN	6		Wood Co., WI	6
	Beckwourth Pass, CA	13	Adams, WI	12	
	Spring Creek, SD	19	Burnett Co., WI	6	
	Ward Mt., NV	6	Warner Mts. (54)	Cedar Pass, CA	4
	Montague, CA	6		Eagle Peak, CA	30
	Gazelle, CA	15		Emerson Peak, CA	20
	Sierra Valley, CA	13			
	Verdi, NV	31			
	Gardnerville, NV	26			
	Boyd Farm, CA	12			
Cedarville, CA	13				
Brandon, SD	20				

Populations are grouped according to their nominal taxonomic designation. Populations designated as alpine are an unnamed subspecies of *L. melissa* occurring above treeline (alpine) in the Sierra Nevada and White Mountains. Populations in the Warner Mountains are referred to the Warner Mts. Entity and are not given species or subspecies designations as their wing pattern morphology is variable.

melissa due to mitochondrial introgression, but they are ecologically and genomically similar to *L. m. samuelis* populations east of Lake Michigan.

Because male genitalic morphology provides the foundation for the taxonomy of the group, we examined male genitalic variation in 868 individuals from 61 populations across North America to address the following questions: 1) Is our genitalic data congruent with Nabokov's species and subspecies designations? 2) Do the suture zones identified from genetic data correspond to areas of intermediate genitalic morphology, or where morphology is incongruent with nominal taxonomy? and 3) If so, is there any morphological evidence of contemporary gene flow between *L. idas* and *L. melissa* at the suture zones, which would be recognized by increased variance in male genitalic morphology compared with populations outside of the suture zone?

Materials and Methods

We obtained male butterflies from 61 *Lycaeides* populations (Table 1) including both fresh and paired specimens. Populations of the endangered *L. m. samuelis* were collected under permit (USFWS permit PRT842392). Specimens were identified to species

and subspecies by using wing pattern phenotypes and geography. Sample sizes for each species and subspecies are provided in Table 1.

The posterior-most abdominal segments of 868 male *Lycaeides* were removed from pinned specimens and from fresh specimens that were subsequently processed for genetic analyses (Nice and Shapiro 1999, Nice et al. 2005, Gompert et al. 2006a,b). Pinned specimens were labeled and stored as vouchers. For those specimens processed for genetic study, wings are preserved in glassine envelopes as vouchers. After submersion in hot ($\approx 100^\circ\text{C}$) 5 M KOH, male abdomens were dissected and genitalia removed under a dissecting microscope. Genitalia for each specimen are stored in labeled micro vials in glycerin and housed in the Lepidoptera Genetics Lab at Texas State University (curated by C.C.N.). 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 (Fig. 2A). Nabokov (1949) used five measurements of male genitalia in his taxonomic revision of *Lycaeides*: falx length (F), humerulus length (H), falx to humerulus length (FH), uncus length (U), and elbow width (E) (Fig. 2A). The FH measurement was excluded here as measurements were inconsistent

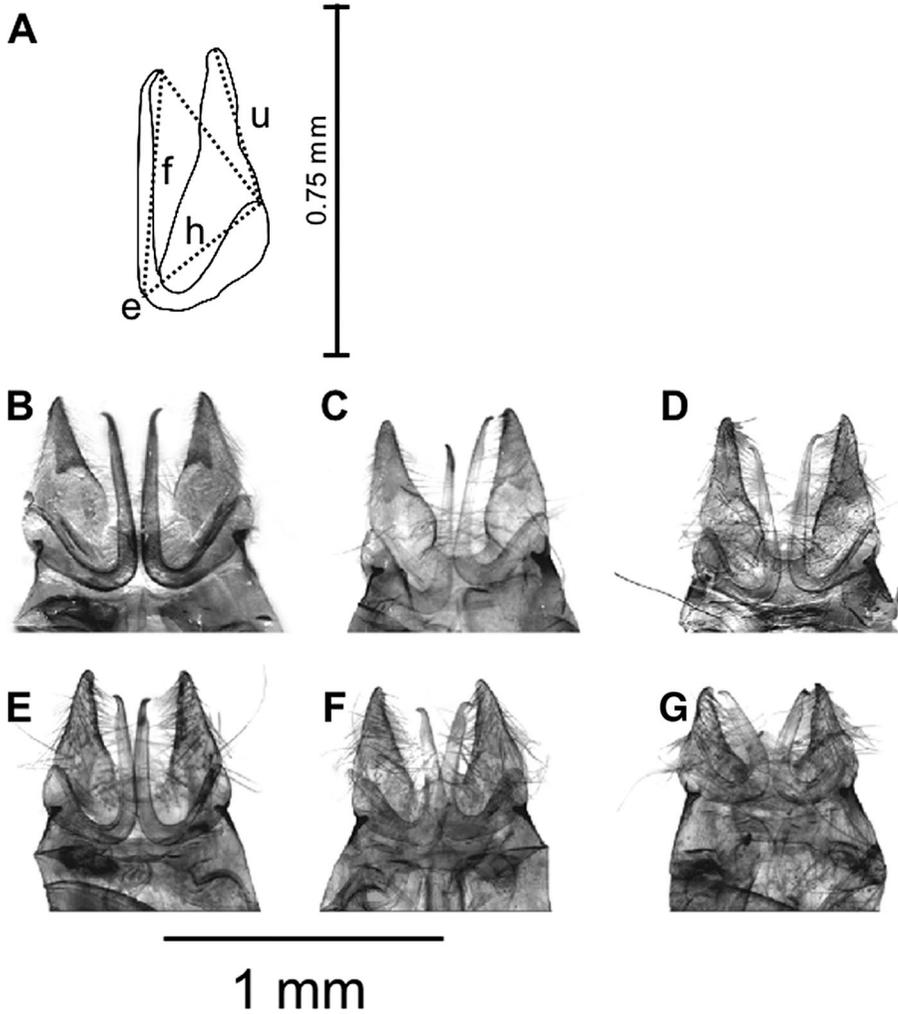


Fig. 2. *Lycaeides* male genitalia. (A) Measurements that Nabokov (1943, 1949) used to delineate *Lycaeides* species and subspecies: falx length (F), humerulus length (H), falx to humerulus length (FH), uncus length (U), and elbow width (E). (B–G) Representative genitalia from each species and alpine populations: *L. melissa* (B and C), *L. m. samuelis* (D), Carson Pass (E), Mt. Rose (F), and *L. idas* (G).

within individuals (i.e., not repeatable). The E measurement also was excluded because there was a lack of precision with this small measurement. A principal component analysis (PCA) was performed to explore morphometric variation and to assess the relative importance of F, H, and U measurements in describing genitalic variation.

Although a PCA was conducted to examine variation within the data set, a canonical discriminant analysis (CDA) was performed to assess aspects of genitalic variation to determine the traits that are most important for discriminating between and among the nominal taxa. Therefore, CDA after multivariate analysis of variance was used to assess the contribution of each trait for discriminating between the two morphospecies and among nominal subspecies. Specifically, we assigned *Lycaeides* to species and subspecies based upon wing pattern and geography, and we used

CDA to assess the reliability of taxonomic delineations of Nabokov (1943, 1949) based on male genitalic morphology. Populations in the Warner Mountains were excluded from the species-level analysis because their species status is uncertain due to variable wing pattern morphology. All individuals from each population in the data set were selected for the subspecies-level CDA, with the Warner Mountains and Sierra Nevada alpine populations treated as separate entities.

To address our question regarding the intermediacy of *Lycaeides* populations around suture zones, means of principal component (PC) 1 scores (which captured ≈87% of the variation) were used to statistically compare suture zone populations, including *L. m. samuelis* populations found in the Great Lakes region in suture zone I, and alpine and Warner Mountains populations found in suture zone VI (Fig. 1A and B), to all other populations grouped by: *L. idas* and *L. melissa*.

Table 2. Principal component (PC) analysis results based on falc (F), humerus (H), and uncus (U) measurements

	PC 1	PC 2
Eigenvalue	2.62	0.31
%	87.38	10.32
Cumulative %	87.38	97.70
Measurements	Loadings	
F	0.60	-0.25
H	0.55	0.82
U	0.58	-0.51

These groupings correspond to the three lineages identified by analyses of genetic variation (Nice and Shapiro 1999; Nice et al. 2005; Gompert et al. 2006a,b), plus the suture zone areas where taxonomic ambiguity occurs. Two alpine populations with small samples sizes were excluded from this analysis. An analysis of variance (ANOVA) was performed on PC 1 scores for all groups to determine whether PC 1 scores differed among groups. *A posteriori* comparisons of means were made using Tukey's honestly significant difference (HSD) test.

One prediction of current gene flow among populations around a suture zone is an excess variance in quantitative characters that is expected in a hybrid swarm caused by the presence of F₂ and backcross individuals (Lynch and Walsh 1998). Variances in male genitalic morphology of suture zone populations were compared with variances from *L. idas* and *L. melissa* populations located some distance from the suspected suture zones (refer to Table 4 for populations used in this analysis). Population variances were calculated for measurements of F, H, and U. Levene's test for equality of variances (Levene 1960, Van Valen 2005) was used to determine whether suture zone I and suture zone VI populations exhibit greater variance in male genitalic morphology than *Lycaeides* populations from

outside this suture zone. Levene's test has the advantage over other methods of variance comparison, such as the F ratio test, that it is robust to deviations from normality (Van Valen 2005). Increased variance in suture zone I or VI populations is interpreted as consistent with the hypothesis of contemporary gene flow between *L. melissa* and *L. m. samuelis*, or *L. idas* and *L. melissa*, respectively.

All statistical analyses were performed using JMP version 5.0.1a (SAS Institute, Cary, NC).

Results

Principal component 1 explained ≈87% of the total variation in male genitalic morphology (Table 2). All measurements (F, H, and U) were roughly equally weighted, and they had positive loadings on PC 1, which indicates that most variation among male genitalia is due to size differences. It is important to note that although *L. melissa* has larger genitalic measurements, the two species are very similar in size (Scott 1986, Opler 1992). If there is any difference between *L. idas* and *L. melissa*, the latter is the smaller species (Opler 1992). Principal component 2 explained an additional 10% of the variation, and it corresponded to a difference in shape (i.e., positive and negative coefficients).

Male genitalic morphology distinguishes North American species of *Lycaeides* with few exceptions (Fig. 2B–G). Approximately 97% of individuals were correctly classified to species: *L. idas* and *L. melissa* (Wilks' Lambda: $F = 1056.38$, $df = 3, 810$, $P < 0.0001$) (Table 3). Means ± SE of the measurements for each species were *L. idas*: $F = 41.99 ± 0.22$, $H = 34.91 ± 0.15$, and $U = 30.36 ± 0.19$; and *L. melissa*: $F = 60.51 ± 0.20$, $H = 39.12 ± 0.10$, and $U = 41.77 ± 0.13$. Canonical coefficients for the three measurements were $F = 0.007$, $H = -0.005$, and $U = 0.004$. All of the 23 indi-

Table 3. Results of CDA based on species (A) and subspecies (B)

A. Species		N		Predicted										
				<i>L. idas</i>				<i>L. melissa</i>						
<i>L. idas</i>		228		99.6				0.4						
<i>L. melissa</i>		586		3.7				96.3						
B. Subspecies		N		Predicted										
				<i>L. i. alaskensis</i>	<i>L. i. anna</i>	<i>L. i. azureus</i>	<i>L. i. nabakovi</i>	<i>L. i. ricei</i>	<i>L. m. annetta</i>	<i>L. m. inyoensis</i>	<i>L. m. melissa</i>	<i>L. m. ? ALPINE</i>	<i>L. m. samuelis</i>	'Warners'
<i>L. i. alaskensis</i>	18	16.7	33.3	16.7	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.2
<i>L. i. anna</i>	98	10.2	56.1	7.1	5.1	18.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1
<i>L. i. azureus</i>	15	20.0	6.7	53.3	6.7	13.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>L. i. nabakovi</i>	26	0.0	0.0	0.0	80.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.2
<i>L. i. ricei</i>	71	9.9	28.2	9.9	5.6	32.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.1
<i>L. m. annetta</i>	15	0.0	0.0	0.0	0.0	0.0	0.0	73.3	0.0	20.0	0.0	0.0	6.7	0.0
<i>L. m. inyoensis</i>	60	0.0	0.0	0.0	0.0	0.0	8.3	43.3	18.3	5.0	25.0	0.0	0.0	0.0
<i>L. m. melissa</i>	185	0.0	0.0	0.0	0.0	0.0	31.4	17.3	42.2	4.9	3.8	0.5	0.0	0.0
<i>L. m. ? ALPINE</i>	94	0.0	0.0	0.0	6.4	0.0	0.0	5.3	2.1	68.1	5.3	12.8	0.0	0.0
<i>L. m. samuelis</i>	232	0.0	0.0	0.0	0.0	0.0	4.3	27.2	4.3	5.2	59.1	0.0	0.0	0.0
'Warners'	54	3.7	3.7	0.0	20.4	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	61.1

Species and subspecies designations (rows) are based on wing phenotype and geography. Predicted species and subspecies (columns) are classified by the discriminant function, based on male genitalia measurements. Classifications are presented as percentages. The bold, diagonal values are the percentage of individuals classified correctly.

Table 4. Mean (variance) F, H, and U measurements for the populations used to test the hypothesis of excess variance in suture zone populations

	A	N	F	H	U	
<i>L. melissa</i> (central clade)						
Ward Mt., NV		6	63.67 (15.07)	40.33 (7.07)	41.33 (7.47)	
Alta, UT		15	65.47 (5.41)	42.00 (1.14)	43.07 (2.78)	
Spring Creek, SD		19	66.11 (4.21)	41.05 (3.72)	43.68 (5.01)	
Brandon, SD		20	67.80 (9.22)	40.80 (3.54)	44.50 (5.00)	
Clay Co., MN		6	63.00 (4.4)	40.00 (11.2)	39.67 (2.27)	
Suture zone I (Western <i>L. m. samuelis</i>)						
Fish Lake, WI		22	61.18 (4.44)	39.36 (2.05)	43.55 (2.64)	
Eau Claire, WI		22	60.82 (6.73)	39.18 (2.92)	43.27 (2.87)	
Black River, WI		18	60.22 (4.65)	39.78 (2.30)	43.56 (2.61)	
Sandhill, WI		21	58.95 (5.85)	38.95 (2.65)	42.67 (3.73)	
Welch/Hartman, WI		10	58.60 (7.16)	38.60 (6.27)	41.80 (3.07)	
Fort McCoy, WI		25	60.16 (4.31)	39.36 (3.24)	43.44 (3.17)	
Necedah, WI		25	59.84 (4.64)	38.96 (2.04)	42.64 (3.91)	
Eastern <i>L. m. samuelis</i> (eastern clade)						
Concord, NH		5	61.60 (4.80)	38.40 (2.80)	45.60 (0.80)	
Saratoga, NY		25	58.32 (3.56)	37.12 (3.69)	43.44 (3.17)	
Allegan, MI		6	59.67 (24.67)	38.33 (7.07)	42.00 (6.40)	
Indiana Dunes, IN		23	59.65 (2.06)	38.00 (3.27)	43.65 (3.15)	
	B	N	F	H	U	Tukey's HSD
<i>L. idas</i> (western clade)						
Shovel Creek, CA		19	42.89 (1.11)	34.89 (1.11)	30.89 (2.11)	B
Indian Valley, CA		15	39.73 (3.35)	32.40 (12.11)	29.2 (8.46)	B
Yuba Gap, CA		25	39.92 (4.83)	35.04 (2.04)	29.12 (2.36)	B
Trap Creek, CA		23	42.17 (5.06)	35.65 (3.15)	29.65 (5.33)	B
Leek Springs, CA		21	39.14 (2.63)	34.19 (4.36)	28.95 (3.05)	B
Donner Pass, CA		20	42.40 (1.94)	35.80 (2.06)	29.40 (8.88)	B
Suture zone VI (alpine populations)						
Carson Pass, CA		36	55.36 (4.64)	39.44 (3.11)	37.44 (4.48)	B
Mt. Rose, NV		24	49.08 (4.86)	36.17 (1.36)	35.00 (3.48)	B
Jeff Davis Peak, CA		12	55.67 (3.52)	39.5 (4.45)	37.5 (3.73)	B
County Line Hill, CA		19	56.63 (17.36)	35.58 (9.15)	38.53 (11.98)	A
Eagle Peak, CA		30	44.73 (2.89)	35.2 (2.37)	31.93 (4.00)	B
Emerson Peak, CA		20	46.70 (3.06)	34.30 (2.22)	31.80 (14.27)	B
<i>L. melissa</i> (central clade)						
Ward Mts., NV		6	63.67 (15.07)	40.33 (7.07)	41.33 (7.47)	AB
Alta, UT		15	65.47 (5.41)	42.00 (1.14)	43.07 (2.78)	B
Spring Creek, SD		19	66.11 (4.21)	41.05 (3.72)	43.68 (5.01)	B
Brandon, SD		20	67.80 (9.22)	40.80 (3.54)	44.50 (5.00)	B
Clay Co., MN		6	63.00 (4.4)	40.00 (11.2)	39.67 (2.27)	B

(A) Results from suture zone I where no significant differences were detected. (B) Results for suture zone VI. Different letters indicate significantly different at $\alpha < 0.05$ (Tukey's HSD).

viduals misclassified occur near or in suture zone VI. This included one individual from Cave Lake (near the alpine populations in the Warner Mountains), one individual from County Line Hill (White Mts.), one individual from Carson Pass (Sierra Nevada), and 20 individuals from Mount Rose (Sierra Nevada). Canonical discriminant analysis correctly classified $\approx 50\%$ of individuals to Nabokov's subspecies delineations (Wilks' Lambda: $F = 134.61, df = 30, 2510.3, P < 0.0001$) (Table 3). The misclassifications at the subspecies level were not limited to populations found along suture zones. Although some subspecies seemed to represent cohesive groups with 60% or more of individuals correctly classified (e.g., *L. i. nabokovi*, *L. m. annetta*, and *L. m. samuelis*), others were much less distinguishable, with fewer than 50% individuals correctly classified (see especially *L. i. alaskensis* with only 16.7% correctly classified) (Table 3B).

All alpine populations examined possess male genitalic morphology intermediate to *L. idas* and *L. melissa*, except the Emerson Peak population in the

Warner Mountains that are not different from *L. idas* (ANOVA: $F = 440.71; df = 8, P < 0.0001$) (Fig. 3). *L. melissa* and *L. m. samuelis* have similar though not identical male genitalic morphology. Populations at Carson Pass (Sierra Nevada), Jeff Davis Peak (Sierra Nevada), and County Line Hill (White Mts.) overlap, and they were more similar to *L. melissa* than *L. idas*. Eagle Peak (Warner Mts.), Emerson Peak (Warner Mts.), and Mt. Rose (Sierra Nevada) overlap, and they were more similar to *L. idas* than *L. melissa*.

Variances of F, H, and U measurements for the 16 chosen populations used to test the hypothesis of excess variance at suture zone I are provided in Table 4A. The variances among populations did not differ (Levene's test: Wilks' Lambda: $F = 0.9302; df = 45, 749.41; P = 0.60$). Suture zone I populations do not contain the excess variance expected from a hybrid swarm, consistent with the hypothesis of no contemporary hybridization. Thus, the western *L. m. samuelis* populations (west of Lake Michigan) are not different from eastern *L. m. samuelis* populations (east of Lake Mich-

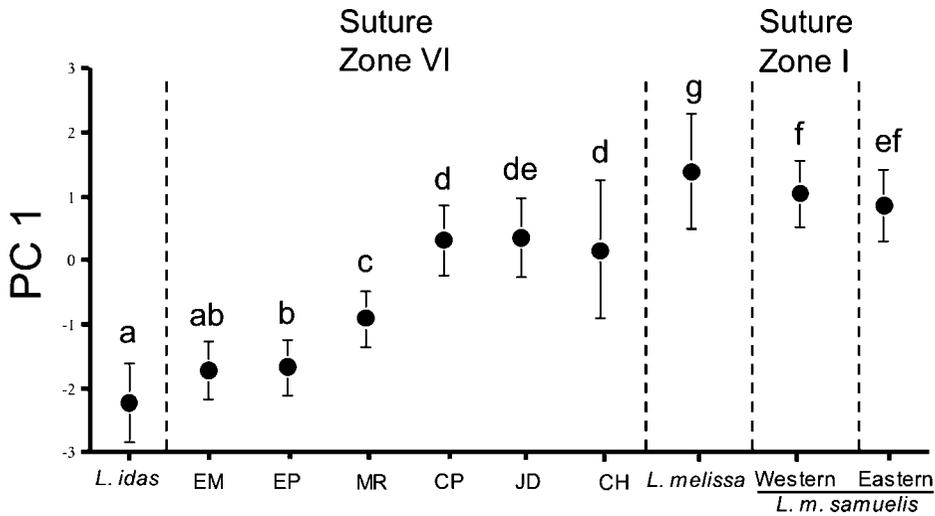


Fig. 3. Principle component scores (mean \pm 1 SD) of male genitalia for *Lycaeides* populations within and outside suture zones I and VI of Remington (1968). Different letters indicate significantly different at $\alpha < 0.05$ (Tukey's HSD). West and east *L. m. samuelis* populations refer to the two mtDNA clades (see text for details). Abbreviated population names for *L. melissa* in suture zone VI are as follows: JD, Jeff Davis Peak; CP, Carson Pass; CH, County Line Hill; MR, Mount Rose; EP, Eagle Peak; and EM, Emerson Peak.

igan), and they are not currently experiencing gene flow from *L. m. melissa*. This supports the conclusion of Gompert et al. (2006b) that gene exchange between *L. melissa* and *L. m. samuelis* has been limited to mitochondrial introgression, with little to no nuclear exchange.

Table 4B provides the data for the 17 populations used to test the hypothesis of excess variance at suture zone VI. The variances among populations differed (Levene's test: Wilks' Lambda: $F = 3.19$; $df = 45, 862.30$; $P < 0.0001$). A posteriori comparisons of means were made using Tukey's HSD test. County Line Hill (White Mts.) was significantly different from all other populations except for the Ward Mountain, Nevada, population. Thus, results from a comparison of variances among populations in suture zone VI are similar to the results from suture zone I. Only a single population in the suture zones exhibits increased variance as would be expected with continuing gene flow. This sole exception at County Line Hill population in the White Mountains does contain excess variance and may constitute a hybrid swarm. Indeed, except for County Line Hill, the three genitalic characters measured were remarkably consistent within populations, and variances were similar for all characters (Table 4).

Discussion

Nabokov's original species and subspecies designations were based on limited sample sizes and lacked statistical rigor (Nabokov 1949, Brown 1950). Our statistical analyses based on a large data set validate the original, morphologically based species designations of Nabokov (1943, 1949). The only individuals

incorrectly identified to species occur in suture zone VI, and they may be of mixed ancestry (Nice and Shapiro 1999, Nice et al. 2005, Gompert et al. 2006a). Conversely, our statistical analyses of male genitalic morphology do not support many of his subspecies designations for North American *Lycaeides*. Moreover, for subspecies, misclassifications were not confined to suture zones. This does not necessarily imply that the subspecies designations are invalid, because wing pattern morphology was also used to delineate subspecies in this genus (Nabokov 1943, 1949), and there are known ecological differences among subspecies (Scott 1986, Nice et al. 2002).

Analysis of PC 1 scores demonstrates that populations at or near suture zones have varying degrees of intermediate morphology between *L. idas* and *L. melissa*. *Lycaeides m. samuelis* is similar to but significantly different from *L. melissa*, which further supports the suggestion that *L. m. samuelis* is a unique, evolutionarily distinct lineage (Nabokov 1989, Cech and Tudor 2005, Gompert et al. 2006b). The intermediate nature of the suture zone VI populations in the Sierra Nevada and adjacent ranges (Warner Mountains and White Mountains) provides evidence of hybrid ancestry, which is also supported by discordance between patterns of genetic variation, morphology and ecological characters at this suture zone (Nice and Shapiro, 1999; Fordyce et al. 2002; Nice et al. 2002, 2005; Forister et al. 2006; Gompert et al. 2006a). Populations of *L. i. nabokovi* also have intermediate morphology, and they warrant further investigation regarding the possibility of hybrid ancestry. Given the number of populations with intermediate genitalic morphology and their location in suture zones, hybridization may have been an important factor in the evolution of North American *Lycaeides* (e.g., Gompert et al. 2006a).

Differences in variance in male genitalic morphology among *L. melissa*, *L. idas*, and the suture zone VI populations were not detected except in the White Mountains of eastern California. Current gene flow among populations around a suture zone should lead to excess variance in quantitative characters as expected in a hybrid swarm due to recombination (Lynch and Walsh 1998). Thus, except in the White Mountains, our analyses indicate no evidence of contemporary gene exchange as recognized by an increase in variance for quantitative traits. Although there may be other explanations for these morphological patterns, molecular investigations corroborate these data and they indicate that there is no evidence of contemporary gene flow in the Sierra Nevada populations (Gompert et al. 2006a). Rather, suture zone VI populations may have arisen by hybridization, but they are not currently experiencing gene flow. The high variance in the White Mountains (County Line Hill) is consistent with the hypothesis that this population constitutes a hybrid swarm. Preliminary data from a detailed survey of mitochondrial DNA support this conclusion (C.C.N., unpublished data).

If gene flow no longer occurs between *L. idas* and *L. melissa* in the Sierra Nevada suture zone VI, as our data suggest, these populations represent a distinct evolutionary lineage or lineages. This conclusion is corroborated by multilocus genetic analyses of suture zone VI populations in the Sierra Nevada that explicitly tested for contemporary gene flow and found none (Gompert et al. 2006a). Indeed, the alpine populations in the Sierra Nevada constitute a distinct species of hybrid origin, which have rarely been documented in animals (Dowling and Secor 1997, Coyne and Orr 2004). This hybrid species possesses a mosaic genome containing alleles from both *L. idas* and *L. melissa*, is differentiated from either of its parental species, and is younger than both (Gompert et al. 2006a). Adaptation to the unique alpine habitat may have led to the evolution of reproductive isolation between Sierra Nevada populations and *L. idas* and *L. melissa*, as suggested by previous investigations (Nice et al. 2002, Fordyce and Nice 2003, Gompert et al. 2006a). The morphological differences described here among lineages add another dimension to the picture of ongoing diversification along suture zones within North American *Lycaeides* that includes a history of gene exchange between differentiated lineages.

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