Egg Morphology Varies Among Populations and Habitats Along a Suture Zone in the *Lycaeides idas-melissa* Species Complex (Lepidoptera: Lycaenidae)

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ABSTRACT   Insect eggs are often characterized by an intricately sculptured external surface, which has been used to a limited extent as a taxonomic character, but not at all in comparisons among populations within a species or among populations of closely related species. We describe egg morphology by using scanning electron microscopy from 12 populations within a species complex of host-specific butterflies in the family Lycaenidae. Variation is found among populations and habitats. Adaptive hypotheses are discussed, and the observed variation is placed within the biogeographical context of the Sierra Nevada Mountains and adjacent regions.

KEY WORDS   biogeography, Remington suture zone, scanning electron microscopy, specialization

The butterfly family Lycaenidae is notable for its richness of species and diversity of natural histories (Grimaldi and Engel 2005). In addition to examples of extreme specialization, in which larvae and adults are associated with a single host plant species, the family contains myrmecophily and instances of carnivorous habits. Lycaenid butterflies have consequently been valuable in studies addressing organismal diversity from a variety of ecological and evolutionary perspectives (Pierce et al. 2002, Lukhtanov et al. 2005, Mouquet et al. 2005). This article concerns a species complex of lycaenid butterflies in North America composed of two nominal species, *Lycaeides idas* (L.) and *Lycaeides melissa* (W.H. Edwards) (Lepidoptera: Lycaenidae) associated with leguminous larval hosts (family Fabaceae) (Fig. 1a). These nominal taxa encompass an array of races and subspecies, including the well-known and federally protected Karner blue butterfly, *L. melissa samuelis*. Members of the *L. idas-melissa* complex have been the subject of intensive investigations into the role of natural selection in maintaining ecologically and morphologically distinct populations (Nice and Shapiro 1999; Fordyce et al. 2002; Nice et al. 2002, 2005; Fordyce and Nice 2003). We herein describe variation among populations of the *L. idas-melissa* complex in the external morphology of eggs as revealed by scanning electron microscopy (SEM) and discuss the biogeographical and ecological contexts in which this variation exists.

Insect eggs are characterized by an often ornate sculpturing, which can serve a number of functions. These functions include protection against desiccation and physical damage, and the facilitation of gas exchange through both structural connections to interior layers of the egg and the maintenance of a plastron, or film of air that allows for respiration when the egg is submerged in water (Hinton 1969, 1970). Our goal is not to address these adaptive aspects of egg morphology directly (although an experiment with eggs submerged in water is described) but to document morphological variation within a portion of the *L. idas-melissa* complex.

We focused on populations in the Sierra Nevada Mountains and adjacent areas of California and Nevada. This region corresponds to suture zone VI in Remington (1968), an area of secondary contact between formerly isolated lineages. Austin and Murphy (1987) called this region a "center of differentiation" where the edges of butterfly species' ranges coincide along a biogeographical boundary, and Swenson and Howard (2004) identified the area as a "hotspot of hybrid zone clustering" (also see Swenson and Howard 2005). A recent survey of mitochondrial DNA (mtDNA) confirms that this region is indeed a location where distinct lineages within the *Lycaeides* species complex meet (Nice et al. 2005). Discontinuities between morphological characters and mtDNA variation in this region suggest that gene exchange has occurred between major lineages (Nice et al. 2005). Populations were sampled in this suture zone from

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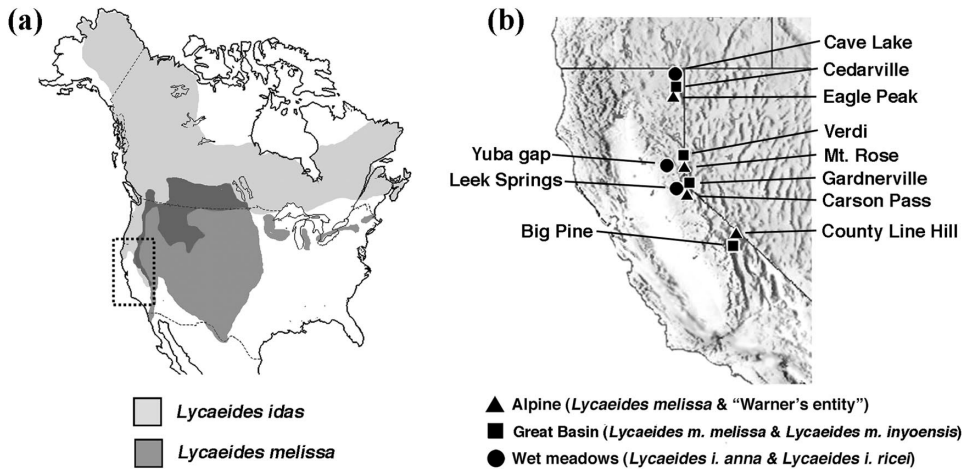
Fig. 1. (a) Distribution of *L. idas* and *L. melissa* in North America, showing area of overlap in the western United States and southwestern Canada; rectangle marks the focal area pictured to the right. (b) Populations from which females and eggs were sampled for the current study; legend indicates habitat and taxa for each location. Great Basin populations and one population in South Dakota, Minnehaha Co. (data not shown), are all associated with alfalfa, *Medicago sativa* L., with the exception of Big Pine, which uses a native host, as do the alpine and wet meadow populations (Table 1).

different habitats and different nominal species and subspecies (Fig. 1b; Table 1). For comparison, a population well away from the suture zone (in South Dakota) also was sampled. Although the potential of egg morphology to inform evolutionary hypotheses has often been suspected in Lepidoptera (Downey and Allyn 1981), this is, to the best of our knowledge, the first comparative study of variation in egg morphology as a population level trait.

Materials and Methods

We obtained a total of 159 eggs from 64 females by confining wild-caught females with host plants. An average of 2.5 eggs per female were preserved at −20C. Sample sizes for the 12 populations are as follows (the number after each population is the number of females from which eggs were studied): Big Pine (three), Carson Pass (seven), Cave Lake (three), Cedarville (five), County Line Hill (five), Eagle Peak (seven), Gardnerville (six), Leek Springs (four), Mt.

Rose (three), SD (six), Verdi (eight), and Yuba Gap (seven). Eggs were mounted on SEM stubs by using carbon tape and sputter coated with gold using a Denton Vacuum Desk II. Each egg was photographed individually, from above, in a Hitachi S3500N scanning electron microscope. See Nickles et al. (2002) for a cross-sectional as well as elemental analysis of eggs of *L. melissa samuelis.*

To characterize variation among populations, principal components were generated from the following measurements taken on each image by using the program NIH Image version 1.62: 1) egg diameter (the mean of four measurements); 2) number of inner ridges (the mean of four counts of the number of ridges in the inner one-third of the egg crossing a transect going out from the center of the micropyle in a random direction); 3) number of outer protuberances (same as measurement two, for the outer one-third of the egg); and 4) diameter of micropyle (the mean of four measurements). The protuberances we refer to are also known as tubercles (Downey and

Table 1. Locations sampled and host plant records

Location	County, state	Latitude/longitude	Host plant
Big Pine	Inyo Co., California	37.17° N 118.29° W	*Glycyrrhiza lepidota* Pursh
Carson Pass	Alpine Co., California	38.58° N 119.83° W	*Astragalus whitneyi* A. Gray
Cave Lake	Modoc Co., California	41.98° N 120.21° W	*Vicia americana* Muhl. ex Willd. (?)
Cedarville	Modoc Co., California	41.53° N 120.17° W	*M. sativa*
County Line Hill	Mono Co., California	37.47° N 118.20° W	*A. whitneyi* (?)
Eagle Peak	Modoc Co., California	41.26° N 120.18° W	*Lupinus polyphyllus* Lindley (?)
Gardnerville	Douglas Co., Nevada	38.87° N 119.77° W	*M. sativa*
Leek Springs	El Dorado Co., California	38.71° N 120.25° W	*L. polyphyllus*
Mt. Rose	Washoe Co., Nevada	39.32° N 119.96° W	*A. whitneyi*
South Dakota	Minnehaha Co., South Dakota	43.63° N 96.54° W	*M. sativa*
Verdi	Washoe Co., Nevada	39.52° N 119.99° W	*M. sativa*
Yuba Gap	Nevada Co., California	39.31° N 120.63° W	*Lotus nevadensis* (S. Watson) E. Greene

Question marks indicate host associations that are unconfirmed.
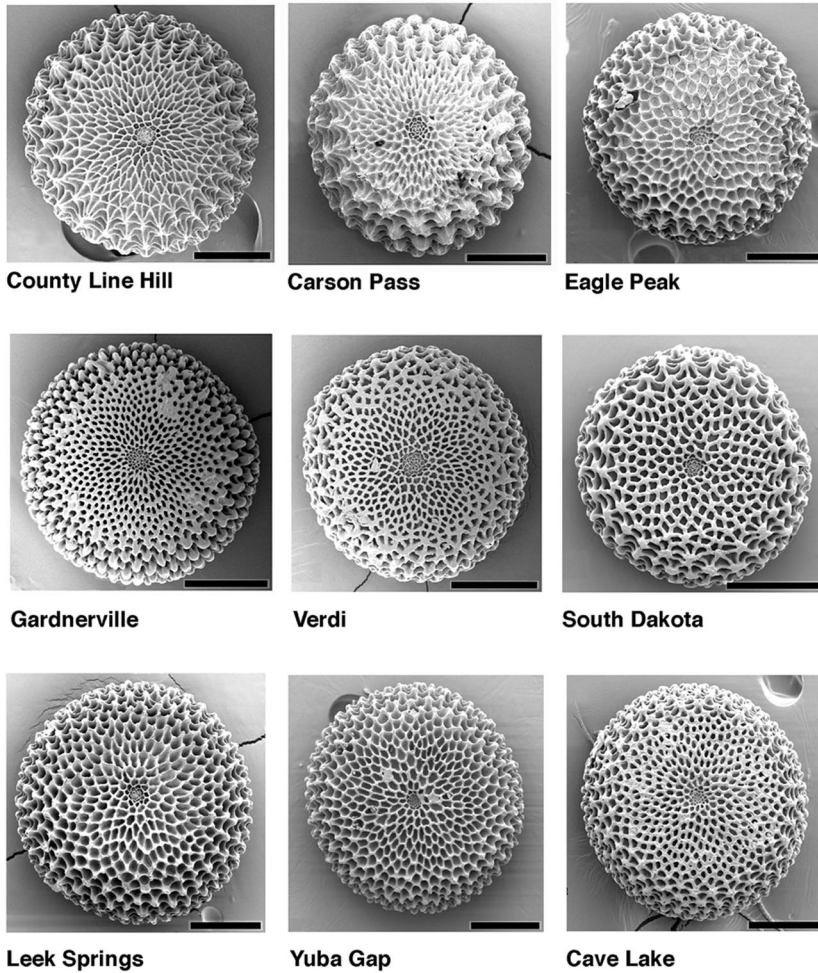
Fig. 2. Representative eggs from alpine populations (top row), Great Basin populations and South Dakota (middle row), and wet meadow populations (bottom row). Scale bars = 250 μm. Average $\pm$ SE egg diameters for each habitat type were alpine, 809.77 $\pm$ 11.79 μm; Great Basin and South Dakota, 710.25 $\pm$ 10.27 μm; and wet meadow, 864.4 $\pm$ 10.5 μm.

Allyn 1981). Values from multiple eggs from each female were averaged before principal component analysis.

Results and Discussion

Variation among populations and habitats was revealed in SEM images (Fig. 2). Eggs from alpine locations were generally characterized by pronounced protuberances on the outer portion of the egg, whereas eggs from wet meadows had a more uniform surface characterized by somewhat deeper cells. Eggs from Great Basin populations tended to have a large number of small protuberances on the distal portion and greater variation in the depth of cells. Despite their geographic distance from the suture zone, SD eggs were similar to eggs from the Great Basin populations (also see Fig. 3). In general, wet meadow eggs were the largest and Great Basin eggs were the smallest (for mean diameters, see Fig. 2 legend). Because

eggs are the stage that overwinters in these populations, egg size may be related to variation in egg provisioning associated with overwintering or a lack thereof, because the smallest eggs are from multivoltine Great Basin populations (had we sampled exclusively from the final generation in Great Basin locations, we might have found larger eggs).

Principal components illustrate some clustering by habitat (Fig. 3) and also reflect the biogeographic context of these populations along the suture zone. The Yuba Gap and Leek Springs populations can be unambiguously assigned to *L. idas* (through genitalic and wing-pattern characteristics) and contain genotypes belonging to a western mtDNA clade as described by Nice et al. (2005). In egg morphology, these populations are distinct from the Great Basin populations (particularly with respect to PC 1; Fig. 3), which can be assigned taxonomically to *L. melissa* and genotypically to a mtDNA clade covering the central United States (from Lake Michigan to the
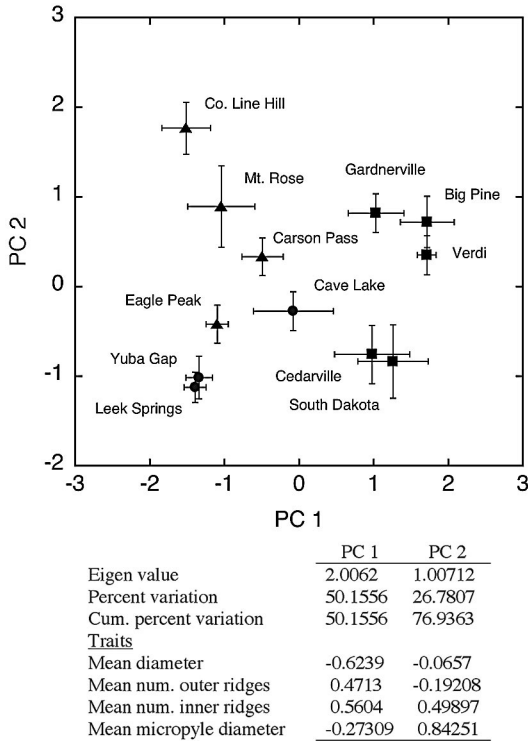
	PC 1	PC 2
Eigen value	2.0062	1.00712
Percent variation	50.1556	26.7807
Cum. percent variation	50.1556	76.9363
Traits		
Mean diameter	-0.6239	-0.0657
Mean num. outer ridges	0.4713	-0.19208
Mean num. inner ridges	0.5604	0.49897
Mean micropyle diameter	-0.27309	0.84251

Fig. 3. Graph illustrates variation in egg morphology with two principal components (error bars correspond to standard errors). Symbols for populations are as follows: triangles, alpine; squares, Great Basin and South Dakota; and circles, wet meadow (same symbols used in Fig. 1b). Details of principal component analyses are shown in table, including loadings for the four traits studied (principal components 3 and 4 not shown).

California-Nevada border). Populations sampled from the middle of the suture zone (the alpine populations and Cave Lake from the Warner Mountains) are often characterized by intermediate genitalic and wing pattern morphologies (C.C.N., unpublished data; Nice et al. 2005) and by discontinuities between these morphological traits and mtDNA lineages. Consistent with this pattern, the alpine and Cave Lake populations are also intermediate with respect to egg morphology (Fig. 3).

Previous work with eggs from some of the same populations has described variation in the adhesion of eggs to host plants (Fordyce and Nice 2003). Females from alpine populations do not firmly attach their eggs to the host plants, which seems to be adaptive, because the above-ground portions of the host plants senesce and are blown downslope. The eggs must survive a drop from the host plant into a generally rocky substrate, where the exaggerated protuberances might provide structural protection (Downey and Allyn 1981). Eggs from the wet meadow populations, however, remain attached to hosts and must respire through repeated submersion in water. The cells observable in wet meadow populations could serve to

maintain a larger or more stable plastron (Hinton 1969). We conducted a small experiment with live eggs to test for differences in the ability of eggs from the different habitats to survive submersion in water. Thirty-four eggs from four populations were overwintered for 5 mo in water at 4°C and subsequently allowed to hatch (18 alpine eggs, 10 wet meadow eggs, and 6 Great Basin eggs). This experiment, although involving a small number of eggs, did not support this hypothesis: 60% of the eggs from wet meadow populations survived submersion, 67% from the alpine survived submersion, and none of the Great Basin eggs tested were viable after the water treatment. The eggs from Great Basin populations probably did not survive because they were not in diapause because of their multivoltine life history; however, the important comparison is between the wet meadow and alpine populations.

The populations discussed here are known to differ in a number of ways, not just morphologically, but also ecologically, most notably in host and mate preference (for host plant records, see Table 1). *L. melissa* females from alpine populations express a consistent preference for their natal host in simultaneous choice tests, whereas *L. idas* females tend to prefer either their natal host or the host of the alpine populations (Nice et al. 2002). Both alpine *L. melissa* and *L. idas* males discriminate, based on subtle wing pattern elements, against heterospecific females (Fordyce et al. 2002). Great Basin populations, in contrast, express no preference for either host plants or mates. Although we cannot confirm an adaptive explanation for the variation in egg sculpturing as of yet, the differences described here among populations and habitats add another dimension to the picture of ongoing diversification along a suture zone within the *L. idas-melissa* complex.

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