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LETTER

Variation in butterfly egg adhesion: adaptation to local host plant senescence characteristics?

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Abstract

Most butterflies that overwinter as diapausing eggs have evolved oviposition behaviours where egg placement is near or on perennating portions of their host plant. We describe an alternative strategy used by alpine populations of a Lycaenid butterfly species complex where eggs are not attached strongly to the leaf substrate and subsequently fall off the plant. Other populations occurring at lower elevations attach their eggs strongly to the plant. In the laboratory, we measured the amount of force required to detach eggs from the leaf substrate. Eggs of the non-alpine populations required more than five times the force required to detach eggs of the alpine population. Field surveys of the host plant characteristics used by various populations suggest that easy detachment may be adaptive for alpine populations because, unlike the host plants used by other populations, nearly all of the preceding year's vegetative growth is blown away by strong winds during the winter months.

Keywords

Adaptation, alpine, egg adhesion, host plant senescence, *Lycaeides melissa*, *L. idas*, Lycaenidae, oviposition, variation.

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INTRODUCTION

Females of most butterfly species choose the host plant on which their larvae will emerge and begin feeding by directly attaching their eggs to the host plant (Singer 1984). However, butterflies with life histories that include diapause in the egg stage are often required to use alternative oviposition strategies. The need for such alternative strategies is obvious for temperate species using herbaceous host plants that senesce prior to female oviposition, or will senesce while the eggs are in diapause (Hayes 1982; Wiklund 1984).

Many species that lay overwintering eggs use woody host plants and attach their eggs to the woody portions of the plant. Other species avoid depositing eggs on the plant altogether, rather depositing or dropping them on substrates near the host plant (MacNeill 1964; Wiklund 1984; Scott 1986; Carey 1994). The behaviour of laying eggs on woody plant parts or substrates other than the larval food plant is probably an adaptation to the senescence characteristics of the host plant. Egg attachment to the host plant would be maladaptive if dead host plant tissue is blown some distance from the following year's growth, leaving emerging larvae far from suitable food (Hayes 1982; Wiklund 1984).

Here, we investigated an apparent alternative strategy for butterflies that overwinter as eggs. Specifically, females search out host plants and lay eggs on them, however, in some populations the eggs fall off the plant shortly after oviposition. In this study, we asked the following questions. Do measurable differences of egg adhesion occur among populations? Do the plant species used by different populations affect egg adhesion? Do the characteristics associated with senescence of the above-ground portions of the host plants used by various populations differ in a way that might explain differences in egg adhesion among populations?

MATERIALS AND METHODS

Study system

For 3 years we intensively studied the host plant preferences of populations of the *Lycaeides* species complex (Lycaenidae) in western North America. In North America, two nominal species occur, *L. idas* (Linnaeus) and *L. melissa* (W.H. Edwards), which feed on papilionaceous legumes in California and Nevada. Though determinable by wing pattern variation (Fordyce *et al.* 2002) and male genital

morphology (Nabokov 1949; Nice & Shapiro 1999), genetic variation at 10 allozyme loci and mitochondrial DNA sequence variation fail to distinguish the two (Nice & Shapiro 1999; Nice *et al.* 2002). We consider that *Lycaeides* is best described as a species complex, and use specific Latin binomials here only due to convention. In California and Nevada, three main ecotypes of this group are present. Univoltine populations of *L. melissa* occur in the alpine-steppe habitats above the tree-line. At mid-elevations on the western slope of the Sierra Nevada, univoltine populations of *L. idas* occur in wet meadows and forest clearings. At low elevations east of the Sierra Nevada, multivoltine *L. melissa* populations feed primarily on agricultural alfalfa.

Over the course of conducting oviposition preference experiments, we noted that eggs of the alpine populations, but not others, were often found on the floor of the experimental arenas. We did not observe any obvious differences in egg size or shape among these populations. Further examination revealed that females were laying eggs on host plants, but they were detached by females crawling on the plants, or by us while we were assessing preference. Figure 1 shows the pattern for eggs observed off the plant over the course of our studies. Although not amenable to statistical analysis because the design of the experiments differed and because we were increasingly careful not to detach alpine eggs, the overall pattern that motivated the present study is evident.

Measuring egg adhesion

Female butterflies were collected from four focal populations: alpine *L. melissa* from Carson Pass, CA (38°58′ N, 119°83′ W, 2900 m), mid-elevation *L. idas* from Yuba Gap, CA (39°19′ N, 120°35′ W, 1700 m) and Leek Springs, CA (38°71′ N, 120°25′ W, 2100 m), and lower-elevation alfalfa-feeding *L. melissa* from near Gardnerville, NV (38°50′ N, 119°47′ W, 1500 m). Individual females were confined in an arena (*c.* 2000 cm^3) consisting of an air-spun polyester mesh bag (Kleen Test Products, Brown Deer, WI) attached to a circular cardboard base and provided various host plant species on which to lay eggs. After 48 h, one to three eggs from each female per host plant species were collected by carefully removing the leaf on which it was attached. Each leaf was firmly attached on either side of the egg (at least 1 cm from the egg) with clear tape to a glass microscope slide.

We estimated egg adhesion as the force required to detach the egg from the leaf surface. We used airflow directed out of a 0.9-mm^2 circular nozzle positioned perpendicularly 1 mm from the egg. Airflow was gradually increased at a consistent rate using a regulator with a pressure gauge. The pressure (N/m^2) was recorded when the egg detached from the leaf surface. The product of the pressure and the area of the egg exposed to the airflow (approximately $3.23 \times 10^{-7} m^2$) provided an estimate of the force (N) required to detach the egg.

Host plant senescence characteristics

Field surveys of host plant condition were conducted in May (low- and mid-elevation sites) or June (alpine) and October of 2001 and June of 2002 to estimate the amount of senesced plant material that remained at the base of the plant. The host plants used by each population are as follows: Carson Pass (*Astragalus whitneyi* A. Gray), Yuba Gap (*Lotus nevadensis* S. Watson), Leek Springs (*Lupinus polyphyllus* Lindley), and Gardnerville (*Medicago sativa* L). All of the host plants used by these populations are perennial herbs. The condition of the first 10 host plants encountered along five randomly chosen transects was assessed. For the spring surveys, we visually estimated the percentage of the previous year's stems and foliage that remained intact at the base of new growing stems. For the autumn survey, the percentage remaining of the current year's growth was estimated in a

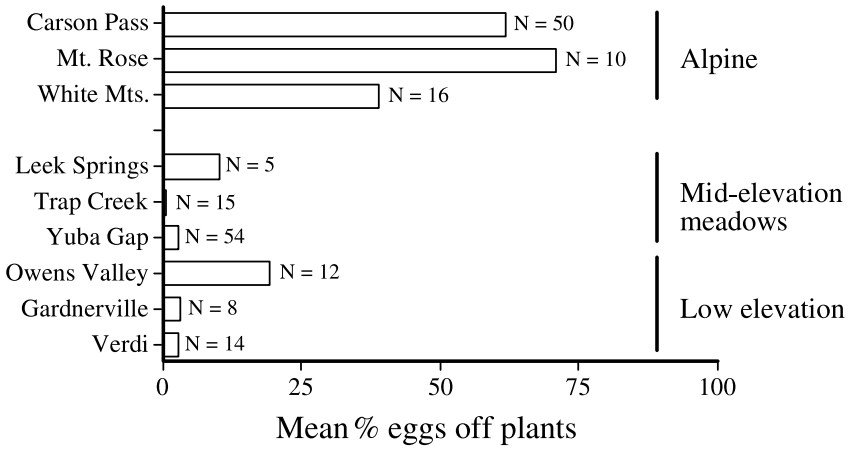

Figure 1 The percentage of eggs observed off the host plants over 3 years of study on the oviposition preferences of *Lycaeides* populations. Numbers adjacent to bars indicate number of experimental arenas over the course of the study.

similar manner, except here we restricted our estimates to the condition of green stems. These data were used to describe the senescence characteristics of the host plants used by these populations.

RESULTS

Egg adhesion

The average amount of force required to detach eggs laid by females from each of the focal populations is summarized in Table 1. The values provided from the Carson Pass population probably represent an upper estimate of egg adhesion because we excluded many eggs that detached from the leaf either when we were removing the leaf from the plant or attaching it to the slide; this never happened with the other populations. Conversely, the adhesion of the remaining populations probably reflects a lower estimate because some eggs withstood the maximum force applied and at times detached the epidermis from the leaf when they did move. The force in these situations was recorded as the maximum (49.0×10^{-3} N). In total, the adhesion of 225 eggs was measured.

Strong host plant discrimination of some females and logistical and phenological constraints of obtaining all host plants for tests on each population prevented us from analysing population and plant effects in a full factorial model. We did, however, have sufficient sample size to compare egg adhesion among Carson Pass, Yuba Gap, and Gardnerville populations on two host plants (Table 2). On both *A. whitneyi* and *M. sativa*, the egg adhesion of the non-alpine populations was more than five times greater than the egg adhesion of the alpine population at Carson Pass. Singer (2002) suggested that the haphazard selection of plants used in this type of experimental design may introduce difficulties in interpreting female preference, because it ignores intraspecific variation in plant acceptability. We can not be certain that similar difficulties do not exist for measurements of egg adhesion. However, eggs have been observed falling off *A. whitneyi* in the field following natural oviposition. Furthermore, independent ANOVAs on egg adhesion within each population failed to detect a plant effect on egg adhesion (not shown).

Table 1 Summary statistics of populations, plant species, number of females, and the average force required to detach eggs from plant

Population	Plant species	*N*	Force† (mean ± SE)
Alpine (*L. melissa*)			
Carson Pass	*Astragalus whitneyi*	13	6.14 ± 3.58
	Lotus nevadensis	1	4.45
	Medicago sativa	6	2.65 ± 0.39
Mid-elevation (*L. idas*)			
Yuba Gap	*Astragalus whitneyi*	11	33.72 ± 2.69
	Lotus nevadensis	20	27.12 ± 2.64
	Medicago sativa	6	20.22 ± 2.21
	Lupinus polyphyllus	4	22.82 ± 8.73
Leek Springs	*Lotus nevadensis*	4	18.37 ± 4.84
	Medicago sativa	3	15.58 ± 1.93
	Lupinus polyphyllus	5	22.26 ± 5.06
Great Basin (*L. melissa*)			
Gardnerville	*Astragalus whitneyi*	6	32.65 ± 3.74
	Lotus nevadensis	1	46.01
	Medicago sativa	9	34.78 ± 3.10

†Force (Newtons $\times 10^{-3}$) required to detach eggs.

Host plant senescence characteristics

Surveys of the host plant phenology and senescence characteristics showed apparent differences among populations. The alfalfa used by the populations near Gardnerville is mowed for hay at various times of the year, preventing us from estimating the amount of dead plant material from the previous year that remains in the agricultural fields. However, *Lycaeides* is more abundant on the uncropped 'feral' alfalfa occurring on roadsides than in monocultures.

Table 2 Analysis of variance for population comparisons of egg adhesion measured as the force (N) ($\log_{10}$ transformed) required to detach eggs from the leaf. Females were treated as a random factor nested within population. The alpine population at Carson Pass required significantly less force to detach eggs from both plant species compared to the non-alpine populations at Yuba Gap and Gardnerville (planned comparisons at $\alpha = 0.05$ with Bonferroni correction)

Test plant	Source	d.f.	MS	*F*	*P*
A. whitneyi	Population	2	0.000593	37.53	< 0.001
	Female(Population)	27	0.000016	1.00	0.4836
	Error	44	0.000016		
M. sativa	Population	2	0.000599	123.11	< 0.001
	Female(Population)	21	0.000005	0.26	0.9992
	Error	35	0.000019		

More than 80% of the previous year's above-ground biomass remains the following spring for these roadside plants, measured in terms of the number of stems from the previous year that remain intact. Some alfalfa varieties grown in mild winter climates retain green shoots above ground all winter (Hanson & Davis 1972), but at Gardnerville this is not the case. The plants used by the Yuba Gap and Leek Springs populations (*L. nevadensis* and *L. polyphyllus*, respectively) also die back each autumn. However, we estimate that more than 80% of the previous year's vegetative plant material remains the following spring, anchored in the soil where the new stems emerge.

The senescence characteristics of the alpine host plant, *A. whitneyi*, differ greatly from the host plants used by the other populations. By the end of October, nearly 50% of its above-ground biomass disappeared, presumably blown away by winds often exceeding 50 km/h (personal observations). We estimate that less than 15% of the preceding year's above-ground biomass is present in spring. In other words, the new growth present in June remains over the summer, begins to disappear by October, and by the following spring nearly all of the previous year's growth is absent. In the spring of 2002, we carefully inspected the 50 *A whitneyi* encountered along our transect and found larvae on 13 plants. The amount of the previous year's plant material did not predict the presence of larvae (logistic regression; d.f. $= 1$, $\chi^2 = 1.55$, $P = 0.21$), nor was there a correlation between the amount of the preceding year's growth and number of larvae observed for plants with larvae (Spearman rank correlation; $P = 0.91$).

DISCUSSION

Female butterflies laying eggs that overwinter in diapause have to contend with the problem of host plant senescence. Species that use woody plants can circumvent this problem by laying eggs on the woody portion of plants that are likely to remain intact through the winter (Hayes 1982; Carey 1994). However, species that use herbaceous host plants often are required to lay eggs on non-host plant substrates, or at the base of the host plant, if it does not remain intact over the winter (Wiklund 1984; Carey 1994). In the *Lycaeides* species complex, we have discovered an alternative strategy that is not determined by the oviposition site choices of the female, but depends instead on a modification of egg adhesion. As such, this alternative strategy appears to be a habitat-specific form of local adaptation (Thompson 1999; Gandon 2002).

The weak egg adhesion observed for the alpine population has obvious benefits, because eggs firmly attached will probably be blown away with the senescing plant material. Unlike the plants used by the other populations, which form large dense patches where the butterfly is present, *A. whitneyi* usually occurs as a solitary plant, often separated by several metres from its nearest neighbour. Conversely, most of the previous year's biomass of *L. nevadensis*, *L. polyphyllus*, and *M. sativa* is always nearly in contact with new spring growth. Thus, eggs attached firmly to the previous year's growth will hatch near a suitable host plant.

Weak adhesion of eggs in alpine populations may represent an adaptive strategy insuring that newly emerged larvae the following spring will be in close proximity to their host plant. When eggs fall off the plant, a natural sifting process occurs which causes them to gravitate to crannies among the litter and soil particles at the base of the plant. Although ants are abundant in the habitat and tend larvae, they do not appear to molest *Lycaeides* eggs (J.A.F. personal observations). Why the alpine *Lycaeides* do not lay directly on the substrate around the base of the host plant is unknown. Natural selection may have acted on egg adhesion if oviposition site is constrained by the need for tactile or chemical stimuli from the host (Renwick & Chew 1994).

The interpopulation egg adhesion differences observed is probably an adaptation to the senescence characteristics of each population's host plant. We do not yet know to what extent this trait is under genetic control. Furthermore, we do not know if the observed differences in egg adhesion reflect differences in female oviposition behaviour (i.e. if females control adhesion) or differences in physiological or morphological traits of the egg. Regardless, the egg adhesion differences observed among populations, coupled with the senescence characteristics of each host plant species, describe an alternative oviposition strategy for butterflies that overwinter as diapausing eggs.

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