The significance of wing pattern diversity in the Lycaenidae: mate discrimination by two recently diverged species

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Abstract

Closely related species of lycaenid butterflies are determinable, in part, by subtle differences in wing pattern. We found that female wing patterns can act as an effective mate-recognition signal in some populations of two recently diverged species. In field experiments, we observed that males from a *Lycaeides idas* population and an alpine population of *L. melissa* preferentially initiate courtship with conspecific females. A morphometric study indicated that at least two wing pattern elements were important for distinguishing the two species: hindwing spots and orange crescent-shaped pattern elements called aurorae. We deceived male *L. idas* into initiating courtship with computer generated paper models of heterospecific females when these pattern elements were manipulated, indicating that the wing pattern elements that define the diversity of this group can be effective mate recognition signals.

Introduction

The great diversity of pattern and colour found on the wings of butterflies inspired much early evolutionary thought (Bates, 1862; Wallace, 1865; Müller, 1878; Darwin, 1880). Investigators appreciated the striking contrast in wing coloration among species and the subtle variation within species and among closely related species (Wallace, 1865; Darwin, 1880). Since that time, our understanding of the development and evolution of butterfly wing patterns has greatly increased. The wing pattern and coloration exhibited by many butterfly species is genetically controlled and complex (Nijhout, 1991; Carroll *et al.*, 1994; Koch *et al.*, 1998; Brakefield & French, 1999). Some species of butterflies exhibit phenotypic plasticity of wing coloration and pattern in response to environmental cues, such as temperature and day length (Shapiro, 1976; Brakefield *et al.*, 1996; Brakefield & French, 1999), and in response to larval hostplant (Knüttel & Fiedler, 2001).

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The functional significance of wing coloration and pattern has been investigated for many butterflies and many adaptive hypotheses, including thermoregulation (Watt, 1968; Kingsolver, 1985; Van Dyck & Matthysen, 1998), crypsis (Scoble, 1992), and inter- and intraspecific signalling have been proposed. Considering the conspicuous appearance of many butterfly wings, it is not surprising that their functioning as signal mechanisms has received the most attention. For example, wing coloration has been shown to be an effective aposematic signal (Brower, 1958; Platt & Brower, 1968; Ohsaki, 1995; Uesugi, 1996; Kapan, 2001). Conspicuous patterns, coloration and melanization of butterfly wings have also been found to function as mate recognition signals (Crane, 1955; Stride, 1957; Lederer, 1960; Silberglied, 1984; Wiernasz, 1989, 1995; Wiernasz & Kingsolver, 1992; Vane-Wright & Boppre, 1993; Warzecha & Egelhaaf, 1995). For example, Jiggins *et al.* (2001) found that the conspicuously different morphs of a *Heliconius* species mate assortatively. Additionally, ultraviolet reflectance patterns of butterfly wings can be important for inter- and intraspecific mate discrimination (Obara, 1970; Rutowski, 1977, 1981; Silberglied, 1984; Brunton & Majerus, 1995; Knüttel & Fiedler, 2001). Despite these examples, subtle differences in pattern are not widely

believed to play a significant mate-signalling role (Scoble, 1992). All the studies of mate recognition mentioned above have investigated the significance of relatively conspicuous differences between species. Here, we ask whether the comparatively subtle differences between very closely related lycaenid butterflies are recognized and utilized as interspecific or even intraspecific mate recognition characters.

Taxonomists often discriminate among closely related butterfly taxa using subtle differences in wing pattern detail. This is especially true for the family Lycaenidae. Commonly called blues, hairstreaks, metalmarks and coppers, the Lycaenidae is the most species-rich group of butterflies comprising between 30 and 50% of true butterflies (Papilionoidea) (Robbins, 1982). However, the functional significance of the complex patterns characteristic of lycaenid wings has largely been unexplored.

In this study, we examined whether the wing pattern differences within a North American lycaenid species complex act as effective mate recognition signals that may contribute to assortative mating. *Lycaeides idas* (Linnaeus) and *L. melissa* (W. H. Edwards) are broadly sympatric across much of North America (Scott, 1986). Genetic variation at 10 allozyme loci and mitochondrial DNA sequence variation fails to distinguish the two, suggesting that they are recently diverged (Nice & Shapiro, 1999). However, these butterflies are determinable by differences in wing patterns (Fig. 1a) and male genitalic morphology (Nabokov, 1949; Nice & Shapiro, 1999) making them 'good' morphological species. They also maintain important ecological differences. In the western USA, *L. idas* occupy mid-elevation, wet meadow habitats. *Lycaeides melissa* occupies much drier habitats: alpine steppe and cultivated agricultural alfalfa (*Medicago sativa*) fields. Both species mate on or near their host plants. Courtship behaviour begins with males patrolling (Scott, 1974) nearly continuously throughout the flight season, searching for stationary females. Stationary females assume a characteristic head-down posture with their wings held closed exposing the ventral hindwing surface (Pellmyr, 1982). Males approach and flutter around stationary females; this initial approach is followed by a series of stereotypical courtship behaviours that lead to copulation (Lundgren & Bergstrom, 1975; Pellmyr, 1982).

Through a series of field experiments we assessed whether males could distinguish between females of the two morphospecies based upon wing pattern phenotype. We predicted that if wing patterns are an important signal for mate recognition, males should show a preference for females of the same morphospecies. Additionally, we conducted a morphometric study to determine what aspects of wing pattern detail explained most of the phenotypic difference between the two morphospecies. To test whether these differences were important for mate recognition, we digitally manipulated the wing pattern
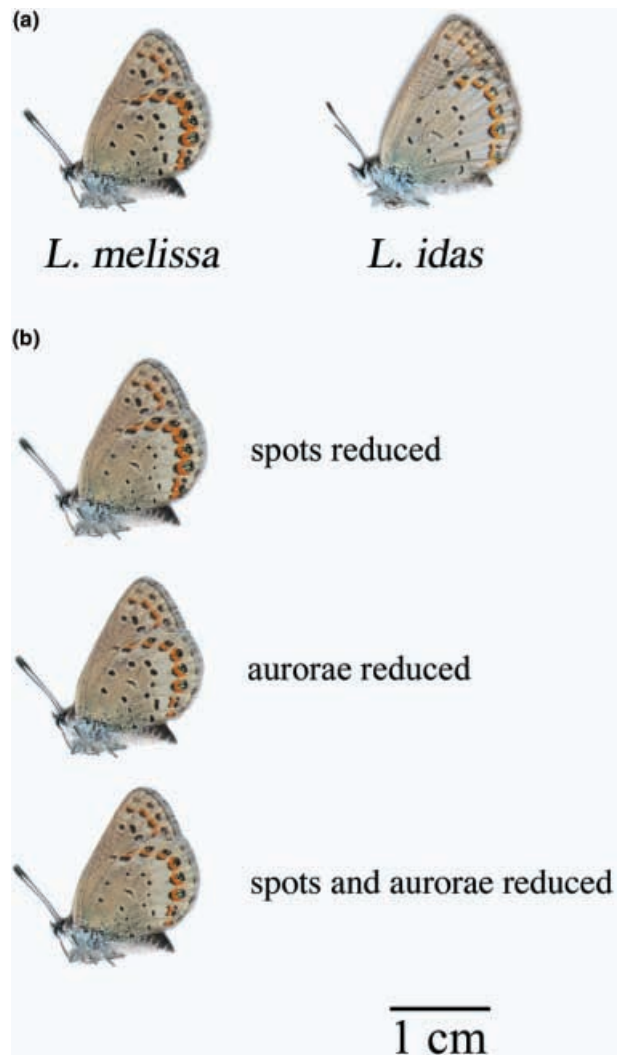

Fig. 1 (a) Female *Lycaeides idas* and *L. melissa* (alpine). (b) The three *L. melissa* female manipulations designed to have '*L. idas*-like' wing patterns.

elements of paper model females of one morphospecies to appear like the other in an attempt to deceive males into courting the 'wrong' females. Understanding the functional significance of the subtle differences in wing patterns that exist among taxa in the Lycaenidae will contribute to our understanding of the diversity of this group, especially when neutral molecular markers fail to distinguish closely related species.

Materials and methods

Population and morphospecies discrimination by patrolling males

We exploited the male approach behaviour towards stationary females to assess female attractiveness to

patrolling males by observing approaches towards dead female decoys. This approach behaviour is equivalent to Phase 1–Phase 2 of the *Lycaeides* courtship described by Pellmyr (1982), which consists of the male fluttering for several seconds within 5 cm around a stationary female. The use of dead female decoys in this manner has been shown to be effective at initiating courtship behaviour for *Lycaeides* in previous studies (Pellmyr, 1982). The populations used in this study were from three locales: *L. idas* at Leek Springs, California (38°71′N, 120°25′W, 2100 m), an alpine *L. melissa* population at Carson Pass, California (38°58′N, 119°83′W, 2900 m) (hereafter referred to as 'alpine-*melissa*'), and *L. melissa* from an alfalfa-feeding population near Gardnerville, Nevada (38°87′N, 119°77′W, 1500 m) (hereafter referred to as 'agricultural-*melissa*'). All of the experiments described herein were conducted between 11:00 and 15:00 hours local time under conditions of full sunlight.

To test the hypothesis that males can recognize and discriminate among females, an experiment was conducted at each population where males were presented with a triangular choice array with three dead females, one from each study population. All females used in each experiment were fresh, unworn and wild-caught, frozen in the field on dry ice, and used within 48 h of capture. Each female was glued with Instant Krazy® Glue Gel (Elmer's Products Inc., Columbus, OH, USA) 1 cm below the tip of 25 cm long wooden skewers. Each female was presented in the head-down position characteristic of stationary females observed in the wild, with wings closed to expose the ventral surface. Each individual was given a number and the length of the left forewing was measured from the thorax to the wing tip with digital calipers to test for a correlation of female wing size with male preference. The three specimens used in each trial were placed among hostplants approximately 30 cm apart, arranged randomly in an equilateral triangle. Each female was used only once and each trial was carried out at a different location within the area occupied by the population. The number of male approaches towards each female in an array was recorded for 15 min after the first approach. Approaches were scored when males altered their flight path and fluttered near a female decoy in the stereotypical behaviour previously described. The number of trials for each experiment was: 16 at the *L. idas* population, 19 at the alpine-*melissa* population and 16 at the agricultural-*melissa* population. Male preference for females from each population was assessed using a Quade test (nonparametric randomized complete block ANOVA) on the number of approaches observed. The Quade test is an extension of the Wilcoxon signed ranks test and has more power than the familiar Friedman test when the number of treatments or groups to choose among is less than five. One important distinction of the Quade test, compared with the Friedman test, is that each block is ranked according to the size of the sample range and the relative significance of each block is adjusted accordingly by rank (Conover, 1999).

Importance of female wing pattern for male preference

Male discrimination among females cannot alone exclude the possibility that pheromonal or other visual cues initiate courtship behaviour. To determine if other cues in addition to female wing pattern were important for male discrimination, we conducted two field experiments at the *L. idas* population. In the first experiment, males were presented with fresh, unworn *L. idas* and agricultural-*melissa* females and *L. idas* females with ventral hindwing scales removed. Hindwing scales were removed by gently rubbing them off using fingers. The experiment consisted of 17 trials lasting 15 min with females placed in triangular arrays as previously described. Each female was used only once. The number of approaches towards each female by males was analysed using the Quade test. Although this design allowed us to remove the visual cues associated with ventral hindwing pattern, it did not necessarily control for pheromonal cues.

To assess the importance of wing patterns alone and to control for pheromonal cues, we conducted a second experiment at the Leek Springs *L. idas* population where males were presented with dead female decoys and paper model females. Paper model females were constructed using colour laser printed reproductions of three female *L. idas* and three alpine-*melissa* females. Females were scanned with their wings closed using a flatbed scanner and each image was flipped horizontally to generate right and left-sided images. A grey scale standard was scanned simultaneously with the butterflies so that scanner colour bias could be corrected for using the colour curve adjustment option in the Adobe Photoshop® 5.0 software package. This method of colour correction avoids observer error by using the computer program colour curves to correct for unbalanced colour characteristics of the scanned grey scale standard. Colour reproductions were created using a colour laser printer that was calibrated for the same computer used for the colour correction, resulting in reproduced butterflies that best matched the real butterflies. The use of constructed model decoys controls for factors such as pheromones and ultraviolet-reflectance patterns, as these characters are not reproduced by the colour laser printer. We predicted that if visible wing pattern alone acts as a mate recognition signal, males in the field could be deceived into initiating courtship with paper models of conspecific females.

Colour images of the butterflies were cut out, glued together with an Elmers® Washable School Glue Stick (Elmer's Products Inc., Columbus, OH, USA), and mounted on skewers as described above. The experimental design was like those previously described, except here four females were presented to males in the field arranged in a square array. Each array included one dead

and one paper model *L. idas* female and one dead and one paper model alpine-*melissa* female. The position of each skewer was randomized within each array and females and models were each used once. Twelve trials were conducted and the number of approaches by males was analysed using a Quade test.

Morphometrics and digitally manipulated paper model females

Our ultimate objective was to determine the differences in wing phenotype between the morphospecies that may be important signals to patrolling males. To assess wing pattern characteristics quantitatively, we conducted a morphometric study on female hindwing pattern. Females from the *L. idas* population (28 individuals) and the alpine-*melissa* population (26 individuals) were scanned using a flatbed scanner. Thirty-two wing characters were quantified using the public domain NIH Image (1.62) program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The morphological landmarks and pattern elements used for this study are shown in Fig. 2. Measurements included the following 12 straight segment length measurements: terminus of the 2A vein to terminus of Sc vein, c to terminus of M1 vein, c to terminus of Cu2, c to b1 of a3, c to b1 of a6, c to proximal edge of M_1, the distal edge of b4 of a3 to the edge of the wing, the width (anterior to posterior) of b2 of a3 at the widest point, and the length (proximal to distal) at the widest point of b1, b2, b3 and b4 of a3. The area of the following 20 pattern elements was taken for each female hindwing: (spots) c, M, Sc(3), Sc, Rs, M_1, M_2, M_3, Cu_1, $Cu_2 + 1A$, $Cu_2(3)$, 2A, (aurorae) b1, b2, b3 and b4 of a2 and a6. All area measurements were square root transformed for analysis. Multivariate analysis of variance (MANOVA) was carried out using these morphological measurements to establish that *L. idas* and *L. melissa* are determinable based upon these wing characters. Following MANOVA, canonical discriminant function (CDF) analysis was employed to assess the contribution of each trait for discriminating between the two morphospecies at these locations. All statistical analyses for the morphometric study were performed using the JMP® IN 4.0.3 (SAS Institute Inc., Cary, NC, USA) statistical program.

To test for the importance of specific wing pattern elements as mate recognition signals, we altered an alpine-*melissa* female using Adobe Photoshop® 5.0 to determine if we could deceive male *L. idas* into initiating courtship with the *L. melissa* paper model. Based upon the morphometric analysis and controlling for the difference in wing size between the two morphospecies, we reduced the size of the alpine-*melissa*'s hindwing spots by 50% and aurorae by 20% so they would appear '*L. idas*-like'. These pattern elements were copied from the main image. The rubber stamp tool in Photoshop® was
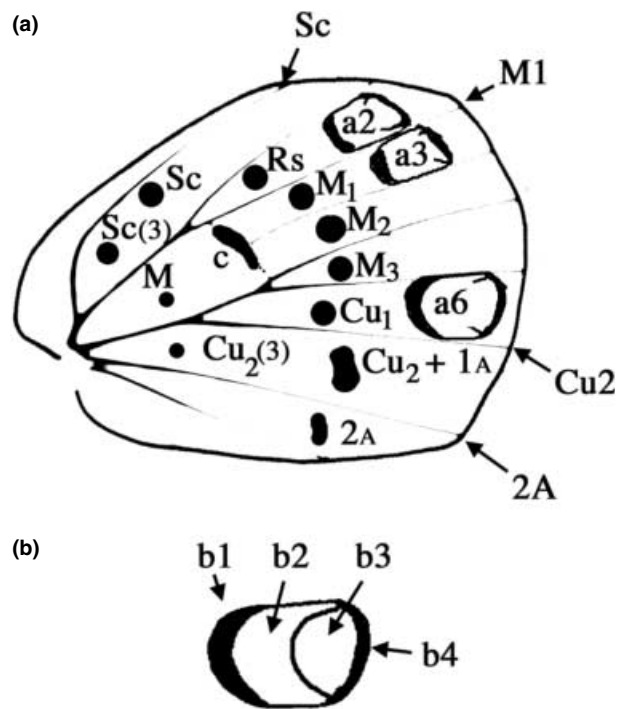

Fig. 2 Schematic depiction of wing pattern elements used for morphometric analysis (see text for details). (a) Ventral hindwing surface of *Lycaeides*. Terminus of selected veins indicated outside of the wing margin. Wing spots synonymous with Nabokov's (1943) 'macule (second)' except for parenthetical 'thirds'. Discodial cell (macule) indicated as *c*. Aurorae (a2, a3, a6) includes Nabokov's (1943) 'semimacule', 'interval I with aurorae' and 'preterminal mark'. (b) Diagram of single aurorae (b1 – semimacule, b2 – orange portion of interval I, b3 – iridescent silver portion of interval, b4 – preterminal mark).

used to remove these elements from the main image and match the adjacent background colour of the wing. The hindwing spots and aurorae, either reduced in size or not, were then placed back on the original image. This gave us the following four model types of *L. melissa*: spots reduced, aurorae reduced, spots and aurorae reduced (Fig. 1b), and unmanipulated (Fig. 1a). Accurate colour reproductions were obtained by colour correction using a scanned grey scale and a colour-calibrated laser printer and paper models were constructed as previously described. Additionally, an unmanipulated female from the *L. idas* population (Fig. 1a) was created in the same manner giving us the total of five model types for the experiment. Six replicate models of each model type were created. The field experiment was conducted at the *L. idas* population. Each of 18 experimental trials consisted of the five model types randomly arranged in a row 0.5 m apart. The individual model of each type used in a trial was randomly chosen. Each trial lasted 30 min and began with the first approach of a male towards a model. A Friedman's test (nonparametric randomized complete

block ANOVA) was used to assess male preference for each of the five models (Conover, 1999).

Results

Population and morphospecies discrimination by patrolling males

Males from two of the three study populations discriminated among the females presented and showed preference for their conspecifics (Fig. 3a). In each of the populations studied, we found no significant correlation between female size and attractiveness to males (Table 1). Males at the *L. idas* population showed a preference for *L. idas* females and showed no difference in preference between *L. melissa* females from either population [number of approaches per trial (mean ± SE), 6.3 ± 0.9] ($T_1 = 13.06$, $P < 0.05$). Alpine-*melissa* males showed a preference for females of *L. melissa* over *L. idas*, however, they did not discriminate between females from the two *L. melissa* populations [number of approaches per trial (mean ± SE), 4.2 ± 0.6] ($T_1 = 6.38$, $P < 0.05$). Unlike the *L. idas* populations and alpine-*melissa* population, agricultural-*melissa* males showed no discrimination among females from the three populations [number of approaches per trial (mean ± SE), 4.8 ± 1.2] ($T_1 = 0.237$, n.s).

Importance of female wing pattern

The result of the ventral hindwing scale removal experiment suggests that factors other than wing pattern alone may be important for mate recognition. Although male *L. idas* showed an overall preference for conspecific females with their hindwing scales intact, they also showed preference for conspecific females with their hindwing scales removed over *L. melissa* females (Fig. 3b) [number of approaches per trial (mean ± SE), 3.8 ± 0.8] ($T_1 = 22.69$, $P < 0.05$). As in the previous experiment, there was no significant correlation between the number of male approaches and female wing length (Table 1).

The experiment consisting of both dead and paper model females was a final effort to test for the importance of wing pattern whilst controlling for other factors, such as pheromonal cues. Males of *L. idas* showed a preference for dead *L. idas* decoys and the paper models of an *L. idas* female over either the decoy or paper model *L. melissa* female (Fig. 3c) [number of approaches per trial (mean ± SE), 6.4 ± 0.9] ($T_1 = 22.10$, $P < 0.05$).

Morphometrics and digitally manipulated paper model females

The MANOVA on the wing characters measured in this study indicated that the species were determinable based upon wing phenotype ($F_{32,21} = 11.90$, $P < 0.001$). The coefficients of the CDF and the mean size of each of the wing measurements are provided in Table 2. Figure 4
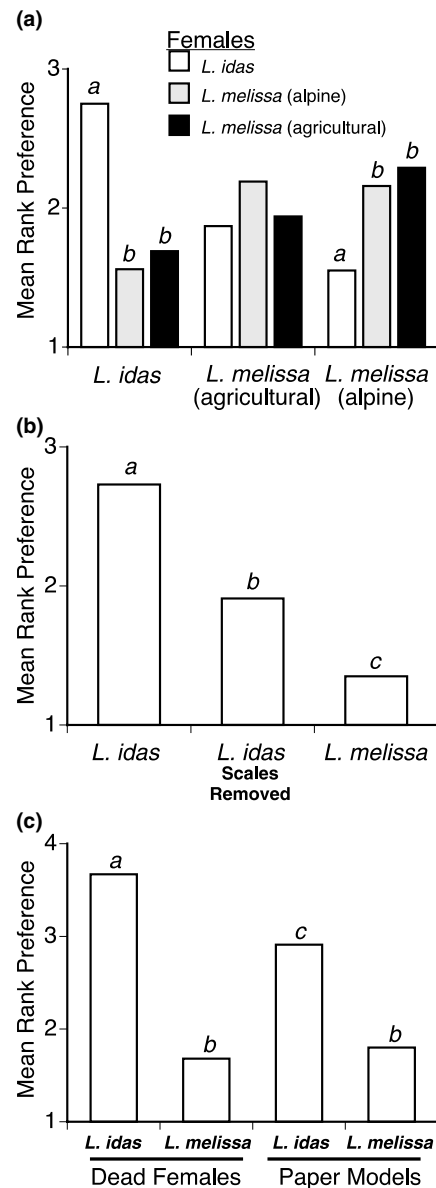

Fig. 3 The mean rank preference of males for the dead or model females presented. Different letters above bars indicate significantly different at $P < 0.05$ significance level (Conover, 1999). (a) Males presented with females from each of the three populations studied. Location of each experiment identified under the *x*-axis. The legend indicates the source population of the females. (b) *Lycaeides idas* males presented with unworn *L. idas* females, *L. idas* females with scales removed, and *L. melissa* females. (c) *L. idas* males presented with dead and paper model females of *L. idas* and *L. melissa*.

graphically illustrates the difference between these two morphospecies based upon the value of the discriminant function (i.e. canonical score) for each butterfly measured in this study.

We successfully deceived patrolling *L. idas* males into initiating courtship behaviour with digitally manipulated

Table 1 Test for correlation between number of approaches by males and female forewing length.

Population	Females	r	P-value*
Alpine – *Lycaeides melissa*	Alpine-*melissa*	0.118	0.67
	Agricultural-*melissa*	0.071	0.80
	L. idas	0.245	0.37
Agricultural – *L. melissa*	Alpine-*melissa*	0.187	0.44
	Agricultural-*melissa*	0.375	0.12
	L. idas	0.195	0.43
L. idas	Alpine-*melissa*	−0.318	0.23
	Agricultural-*melissa*	0.164	0.55
	L. idas	−0.018	0.95
L. idas	*L. idas*	0.271	0.34
	L. idas (pattern removed)	0.319	0.25
	Agricultural-*melissa*	0.414	0.13

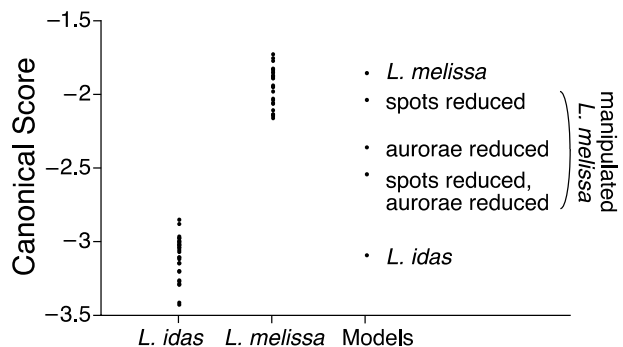*Fisher's *r* to *z* transformation (Sokal & Rohlf, 1995).

L. melissa paper models (Fig. 5) [number of approaches per trial (mean ± SE), 6.8 ± 1.2] ($T_2 = 15.17$, $P < 0.01$). This effect was especially true for *L. melissa* model females that had reduced spot size, where males showed equal preference for the manipulated models and the conspecific model. Figure 4 shows where each of the five models used in this experiment falls within the linear morphospace described by the discriminant function.

Discussion

The results of this study demonstrate that the wing pattern differences that occur between these two morphospecies can act as effective mate recognition cues in some populations. The difference in male discrimination behaviour between the two *L. melissa* populations was surprising and may reflect the ecological and historical differences that occur between these two populations. The alpine-*melissa* population and the *L. idas* population used in this study are potentially in contact at the periphery of their range, whereas the agricultural-*melissa* are disjunct. It is possible that wing pattern recognition may be acting as a reinforcing character (Howard, 1993) for the *L. idas* and alpine-*melissa* population to reduce the flow of genes adapted to the decidedly different habitats these populations occupy. If the agricultural-*melissa* rarely or never encounter heterospecific females, there may be little reinforcing selection for wing pattern recognition. Alternatively, the lack of male discrimination observed in the agricultural-*melissa* may reflect their recent origin. Alfalfa was first introduced to North America in the 1800s (Michaud *et al.*, 1988). If the *L. melissa* that currently use alfalfa throughout North America was established by individuals able to adapt to a wide range of environmental conditions, such as a novel hostplant in an agricultural system, they too may have a wide range of acceptable mate phenotypes. Thus, the lack

Table 2 Canonical discriminant function (CDF) coefficients for each measurement included in the morphometric study and the mean size (untransformed) of each of the morphological characters of *Lycaeides idas* and *L. melissa*.

Measurements	CDF coefficients	*L. idas*	*L. melissa*
Length measurements		Mean (mm) ± SE	Mean (mm) ± SE
2A – Sc	−0.122	8.71 ± 0.08	7.84 ± 0.09
c – M1	0.058	7.12 ± 0.08	6.22 ± 0.07
c – Cu2	−0.490	6.48 ± 0.07	5.61 ± 0.06
c – b1(a3)	−0.413	4.95 ± 0.07	4.01 ± 0.05
c – b1(a6)	0.309	4.18 ± 0.06	3.35 ± 0.05
c – M1	0.004	2.50 ± 0.04	2.07 ± 0.04
b4(a3) – edge of wing	−0.175	0.71 ± 0.02	0.64 ± 0.01
width b2(a3)	−0.237	1.04 ± 0.02	1.04 ± 0.02
b1(a3)	0.048	0.23 ± 0.01	0.26 ± 0.02
b2(a3)	0.268	0.41 ± 0.02	0.48 ± 0.02
b3(a3)	0.304	0.30 ± 0.03	0.55 ± 0.03
b4(a3)	−0.309	0.38 ± 0.04	0.17 ± 0.02
Area measurements		Mean (mm²) ± SE	Mean (mm²) ± SE
Spots c	−0.280	0.23 ± 0.02	0.29 ± 0.02
M	0.753	0.13 ± 0.01	0.24 ± 0.01
Sc(3)	−0.329	0.19 ± 0.01	0.26 ± 0.02
Sc	−0.570	0.22 ± 0.01	0.30 ± 0.02
Rs	−0.140	0.20 ± 0.01	0.31 ± 0.01
M1	−0.631	0.19 ± 0.02	0.29 ± 0.02
M2	0.981	0.23 ± 0.02	0.31 ± 0.02
M3	−0.003	0.13 ± 0.01	0.21 ± 0.01
Cu₁	1.170	0.18 ± 0.01	0.26 ± 0.02
Cu₂ + 1A	−0.455	0.25 ± 0.02	0.34 ± 0.02
Cu₂(3)	0.520	0.09 ± 0.01	0.17 ± 0.02
2 A	−0.260	0.21 ± 0.01	0.24 ± 0.02
Aurorae b1(a2)	−0.356	0.20 ± 0.02	0.24 ± 0.02
b2(a2)	0.792	0.35 ± 0.03	0.55 ± 0.03
b3(a2)	−0.142	0.18 ± 0.02	0.35 ± 0.03
b4(a2)	0.074	0.19 ± 0.02	0.14 ± 0.02
b1(a6)	0.439	0.28 ± 0.02	0.30 ± 0.02
b2(a6)	0.442	0.77 ± 0.36	0.84 ± 0.04
b3(a6)	0.709	0.47 ± 0.03	0.70 ± 0.04
b4(a6)	0.661	0.11 ± 0.01	0.16 ± 0.02

Fig. 4 The canonical score provided by the discriminant function of the each of the butterflies from the *Lycaeides idas* and alpine-*melissa* populations. Included are each of the paper models used in the digital manipulation experiment.
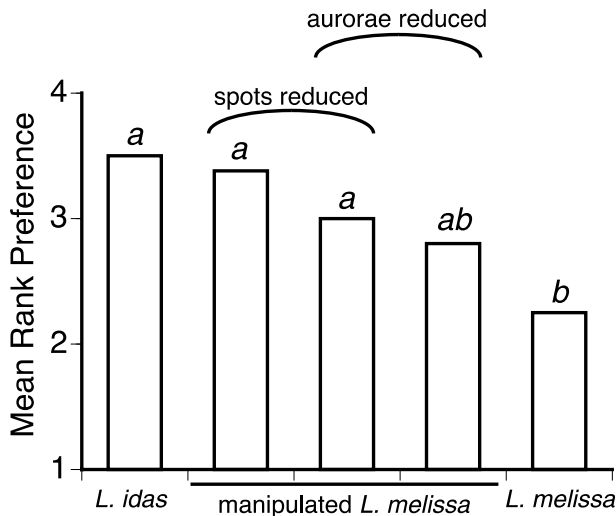
Fig. 5 The mean rank preference of *Lycaeides idas* males towards paper model females. Brackets above the bars indicate the pattern elements manipulated. Different letters indicate rank preference different at a *P* < 0.05 significance level (Conover, 1999).

of the fine scale mate discrimination may be linked to the eurytopic lifestyle of agricultural-*melissa* overall. Unfortunately, we cannot discriminate between these two hypotheses as they are beyond the scope of this study. However, the observed discrimination among females by males in two of the populations studied here indicates that these males recognize and prefer females of the same morphospecies. Wing pattern phenotype may be a character that is under selection in these butterflies, and thus these phenotypic differences may be apparent before lineage sorting at neutral molecular markers has time to occur (Nice & Shapiro, 1999).

The experiment where dorsal hindwing pattern was removed suggests that in addition to hindwing pattern, some other factor not considered also contributes to the attractiveness of females to patrolling males. Although male *L. idas* preferred unworn female *L. idas* over female *L. idas* with wing patterns removed and the *L. melissa* females, the females with wing pattern removed were favoured over the heterospecific females. In many butterflies, courtship behaviour is elicited by visual cues (Silberglied, 1984; Vane-Wright & Boppré, 1993) and pheromones usually release behaviours only over short distances (Lundgren & Bergström, 1975; Silberglied, 1984; Vane-Wright & Boppré, 1993). In *Lycaeides*, pheromone emitting scales have been identified on the dorsal wing surface of males (Lundgren & Bergström, 1975; Pellmyr, 1982). Although trace amounts of volatile compounds have been detected on females' wings, the origin of these compounds remains unknown (Pellmyr, 1982). Thus, pheromones may play some role in male mate recognition for these butterflies. However, Lundgren & Bergström (1975) observed that the pheromones of *Lycaeides* release

the precopulatory behaviour only at short distances after initial phases of courtship are completed, in other words, after the courtship phases observed in this study have occurred. Alternatively, some other aspect of the female morphology may be used in conjunction with wing patterns as a signal to males. Conversely, some aspect of the *L. melissa* phenotype, such as ultraviolet-reflectance patterns, may be unattractive to *L. idas* males and effectively repel their approach. Although it is not known if ultraviolet reflection plays an important role in mate discrimination for *Lycaeides*, it is known to be important for other lycaenid species (Knüttel & Fiedler, 2001), and thus it should not be discounted for this system.

The paper models were used to control for potential pheromonal cues of females. In the experiment where males were presented with dead and paper model *L. idas* and *L. melissa*, the males showed the strongest preference for the unworn dead female. This again supports the possibility that some factor in addition to wing pattern acts as a mate recognition signal. However, they did show a preference for the *L. idas* paper model over both the dead and paper model *L. melissa* females, indicating that the subtle differences observed in the wing patterns of these two species can act as effective mate recognition signals. The use of the Elmers glue stick on both the paper models discounts the potential confounding factor of some characteristic of the glue being attractive because *L. idas* males showed significantly less preference for the *L. melissa* paper females compared with the *L. idas* paper females.

Using the information from the morphometric study, we were able to digitally manipulate two important sources of variation that are quantitatively different between *L. idas* and *L. melissa* to make one species appear more like the other. When *L. melissa* female models were manipulated to appear more '*L. idas*-like', they were more attractive to patrolling males. Artificial models have been used in previous studies investigating the function of conspicuous butterfly wing patterns and colours as mate recognition cues, at least as far back as the seminal work of Crane (1955) and Stride (1957). To our knowledge, however, the digital quantification and manipulation methods used in this study have not been attempted before. This method should be used with caution, however, because the spectral properties of the colour laser printer reproductions may be perceived differently by the butterflies than actual female butterflies are perceived. For example, the orange of the aurorae is rendered by the printer as a combination of colours, presumably yellow and magenta. This may explain why the aurorae manipulation did not elicit the same response as the spot manipulation. It is possible the butterflies perceived the orange of the aurorae differently than the human eye does. Thus, we cannot conclude that ventral hindwing spots are more important than aurorae for mate recognition. However, this experiment did demonstrate that some of the diagnostic differences in wing pattern indicated by the canonical discriminant analysis act as mate

recognition signals because *L. idas* males were attracted to the manipulated *L. melissa* models. That male *L. idas* can discriminate among the models is striking, in light of the subtle pattern manipulations used in this study.

The release of courtship behaviours elicited by visual cues is not entirely novel in the Lycaenidae. Bernard & Remington (1991) found differences in the colour sensitive pigments in the eyes of two closely related species (*Lycaena rubidus* and *L. heteronea*), elucidating a mechanism for mate discrimination. However, courtship behaviours of these species are considerably different from those of *Lycaeides*. Specifically, *L rubidus* and *L. heteronea* females discriminate among potential mates based on wing phenotype but males do not use visual cues to find females, rather they use wind-carried pheromones emitted by the females (Bernard & Remington, 1991). More importantly, the phenotypic differences used for visual discrimination between these two species are not nearly so subtle. Male *L. heteronea* are bright blue and *L. rubidus* are copper coloured. Pellmyr (1982) found that European *L. idas* males could discriminate between conspecific females and females of another lycaenid species, *Plebejus argus*. Although he did not specifically test for the importance of ventral wing patterning, he suggested that it might play a role in male discrimination of females, a suspicion supported by our results.

Our results show that the detailed patterns found on the wings of *Lycaeides* can act as effective mate recognition signals. Subtle differences in wing patterns have been used to describe Lycaenid diversity as have differences in genitalia (Nabokov, 1949). Ascribing a mate signalling function to wing pattern differences not only lends some validity to the taxa, it also brings to our attention the importance of morphological and ecological traits when considering the diversity of this group, especially when molecular markers fail to discriminate among recently diverged taxa. Although not investigated here, the evolution of genitalic differences between *L. idas* and *L. melissa* may also contribute to assortative mating which is not reflected in surveys of molecular genetic variation (Nice & Shapiro, 1999). The conservation implications of such considerations are apparent in light of our inability to identify *L. idas* and *L. melissa* based upon neutral molecular markers (Nice & Shapiro, 1999). Nearly half of all the butterflies currently protected in USA under the Endangered Species Act are in the family Lycaenidae (US Fish & Wildlife Service, 1992). All these protected lycaenids are designated as subspecies, including two from the *Lycaeides* group: *L. melissa samuelis*, the Karner Blue, and *L. idas lotis*, the Lotis Blue, now believed extinct (Arnold, 1993). Morphological variants or taxa with subspecific designations based upon wing patterns, may represent evolutionarily significant units as assortative mating and the evolution of reproductive isolation begins through the modification of wing patterns. Wing pattern morphology, then, may be an early indicator of divergence in this group, and may more accurately describe the diversity of this group than molecular markers alone, especially for taxa that have recently, or are currently, undergoing diversification.

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