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Butterflies show flower colour preferences but not constancy in foraging at four plant species

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Abstract. 1. The extent to which flower colour and other visual cues influence butterfly flower choice in the field is poorly understood, especially in comparison with choices by Hymenoptera.

2. Using a novel approach to studies of visitation behaviour by butterflies, flower colour of four Asteraceae species was phenotypically manipulated to decouple the influence of that trait from others (including morphology and nectar rewards) on visitation by *Lycaena heteronea*, *Speyeria mormonia*, *Cercyonis oetus*, and *Phyciodes campestris.*

3. Flower visits were recorded to experimental flower arrays in subalpine meadows to measure (i) spontaneous preference by butterflies for particular colours and other traits and (ii) flower constancy (longer than expected strings of visits made to flowers of the same species), a behaviour that can reduce interspecific gene flow in plants.

4. Over three field seasons, 3558 individual flower visits in 1386 foraging bouts were observed for free-flying butterflies. All four butterfly species responded to the phenotypic manipulations of flower colour, although in different ways. *Speyeria mormonia* and *L. heteronea* also exhibited preferences based on other flower traits. *Lycaena heteronea* responded to combinations of traits such that the other traits it preferred depended upon the context of flower colour.

5. None of the butterfly species exhibited flower constancy in any of the arrays employed.

6. The observed preferences show that butterflies, like some other pollinators, are potentially capable of exerting selection on colour and other floral traits. Moreover, these flower preferences can depend on the context of other flower traits. The absence of constancy contrasts with reports of high constancy in many bees.

Key words. *Cercyonius*, flower colour preference, Lepidoptera, *Lycaena*, *Phyciodes*, plant–animal interactions, pollinator, *Speyeria.*

Introduction

Insect pollinators display a variety of flower-visiting behaviours that influence pollen dispersal. These behaviours include flower choice, time spent foraging at a nectar source, and number of flowers visited in a foraging bout. A pollinator's choice of flowers depends initially on how flowers are perceived by its sensory system. Visual signals such as flower colour, shape, and size can play an important role in flower detection and choice (Levin, 1968; Waser & Price, 1983). Signals other than visual ones, such as the presence of fragrances and the volume and components of nectar, can also be involved in attracting insects to flowers (Erhardt & Rusterholz, 1998; Wright & Schiestl, 2009).

Flower visitation behaviour has been extensively studied in hymenopteran pollinators, in comparison to flies, beetles, and butterflies (Weiss, 2001; Johnson *et al.*, 2003). Nevertheless, the latter groups can constitute the main pollinator fauna in

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some ecological communities (Arroyo *et al*., 1982; Kearns, 1992). This study focuses on the visual cues that attract adult foraging butterflies to flowers.

Many studies have shown that butterflies have the ability to respond to colour and learn new associations between colour and nectar rewards (e.g. Swihart, 1971; Scherer & Kolb, 1987; Goulson & Cory, 1993; Kandori & Ohsaki, 1996; Kelber & Pfaff, 1997, and references therein; Weiss, 1997; Kinoshita *et al*., 1999), most of them using artificial flowers (but see Weiss, 1995; Neumayer & Spaethe, 2007). Despite this relative wealth of information, the behavioural consequences of colour vision while foraging on real flowers under field conditions are poorly understood. In field studies, demonstrating that colour is the trait butterflies are responding to would require that colour be phenotypically or genetically manipulated (Rausher, 2008). Despite early experiments of this sort by Clements and Long (1923), only a handful of more recent studies have decoupled the effect of colour from other floral traits through trait manipulations in the field, and none of them involved butterflies (Waser & Price, 1983; Peter & Johnson, 2008; for review see Rausher, 2008).

Pollinators can exert selection on plant traits through flower preference and flower constancy, which affect pollen carryover and transfer. Our study was designed to assess flower preference of butterflies in its most general sense, the net over-visitation of one flower type over another. As illustrated by studies with other insects, such behaviour has the potential to generate selection on floral traits (e.g. Galen, 1989). It can, however, result from many causes, including perceptual ability, innate choice, and learning. Flower constancy is the restriction of flower visits to fewer flower types or species than available due to intrinsic limitations in memory (Waser, 1986; Raine & Chittka, 2007a), or learning abilities (Chittka *et al*., 1999). Flower-constant visitors show a propensity to visit flower types previously visited, bypassing alternative, equally rewarding types. Flower constancy has been examined in few Lepidoptera species (Lewis, 1986; Goulson & Cory, 1993; Goulson *et al*., 1997a,b). These studies found high fidelity in the butterflies' choice of flowers, but conflicting results concerning the existence of costs associated with switching from one flower type to another. Moreover, there is little evidence supporting the existence of the memory or other constraints needed for constancy to occur (Lewis, 1989; Kandori & Ohsaki, 1996). None of these studies evaluated constancy using experimental arrays of real flowers in the field, as necessary to measure its strength in natural environments (Waser, 1986). Our intent was to examine whether spontaneous preference and constancy occur under field conditions, where they have the potential to influence selection and gene flow in plants (Meléndez-Ackerman & Campbell, 1998).

We used arrays of phenotypically manipulated flowers to determine if free flying *Lycaena heteronea* Boisduval, *Lycaena rubidus* Behr (Lepidoptera: Lycaenidae), *Speyeria mormonia* Boisduval, *Cercyonis oetus* Boisduval, and *Phyciodes campestris* Boisduval (Lepidoptera: Nymphalidae) butterflies show flower preferences and/or constancy. Using experimental manipulations of flower traits to tease apart the effects of colour and other traits under field conditions, we asked the following questions: (i) Do these butterflies show spontaneous preferences for flower colour and/or for other floral features? (ii) Are these butterflies behaving as constant flower visitors, and does the degree of constancy depend on the choice of traits presented?

Methods

Study system

We worked near the Rocky Mountain Biological Laboratory (RMBL), in Gunnison County, Colorado, on two lycaenid and three nymphalid butterfly species. The ruddy copper (*Lycaena rubidus*) and the blue copper (*Lycaena heteronea*) live in high elevation habitats from British Columbia to North Dakota, and from central California to northern New Mexico. The larval host plants of these species are Polygonaceae from the genus *Rumex* in the case of *L. rubidus* and *Eriogunum* in *L. heteronea* (Glassberg, 2001). The mormon fritillary (*Speyeria mormonia*), the field crescent (*Phyciodes campestris*), and the small wood nymph (*Cercyonis oetus*) are nymphalid butterflies, also widely distributed in mountainous western North America. Their larvae feed on species of *Viola*, *Aster*, and *Machaeranthera*, and various species of grasses, respectively (Glassberg, 2001).

Adult butterflies of the five species feed on the nectar of many different species in the Asteraceae (N. Pohl, pers. obs.; Glassberg, 2001). We chose four species that were primarily visited by butterflies at our sites (N. Pohl, pers. obs.). To assess flower colour independently from the human visual system, we measured the reflectance spectra of unmanipulated and painted flowers with a S2000 Miniature Fiber Optic Spectrometer (Ocean Optics, Dunedin, Florida) over a 300–700 nm range. The mountain yarrow (*Achillea alpicola* Nuttall) has clusters of white florets that evenly reflect wavelengths above 400 nm and lack a strong ultraviolet reflectance (Fig. 1a), whereas the flower heads of the showy fleabane (*Erigeron speciosus* Lindall) have yellow disk flowers and, normally, purple/blue ray flowers with a peak reflectance at 450 nm and a steady increase in reflectance above 650 nm, also without an ultraviolet component (Fig. 1b). In 2007 we also found specimens of *E. speciosus* possessing white ray flowers. All flowers of Hoope's sneezeweed (*Dugaldia hoopesii* Rydberg) are orange, with reflectance rising above 550 nm (Fig. 1c), whereas both ray and disk flowers of mule's ear (*Wyethia amplexicaulis* Nuttall) are yellow, reflecting wavelengths above 500 nm (Fig. 1d). Flowers of *D. hoopesii* do possess ultraviolet reflectance, expressed as a 20% reflectance peak at 350 nm (Fig. 1c).

Experimental arrays

The behaviour of free-flying butterflies was recorded while feeding on artificial arrays of flowers constructed to examine the butterflies' response to colour and other flower cues. Prior experience of these butterflies was not controlled, and so spontaneous flower preference could reflect a combination of perceptual ability, innate preference, and learned behaviour. These arrays were set in the wild near natural populations of the

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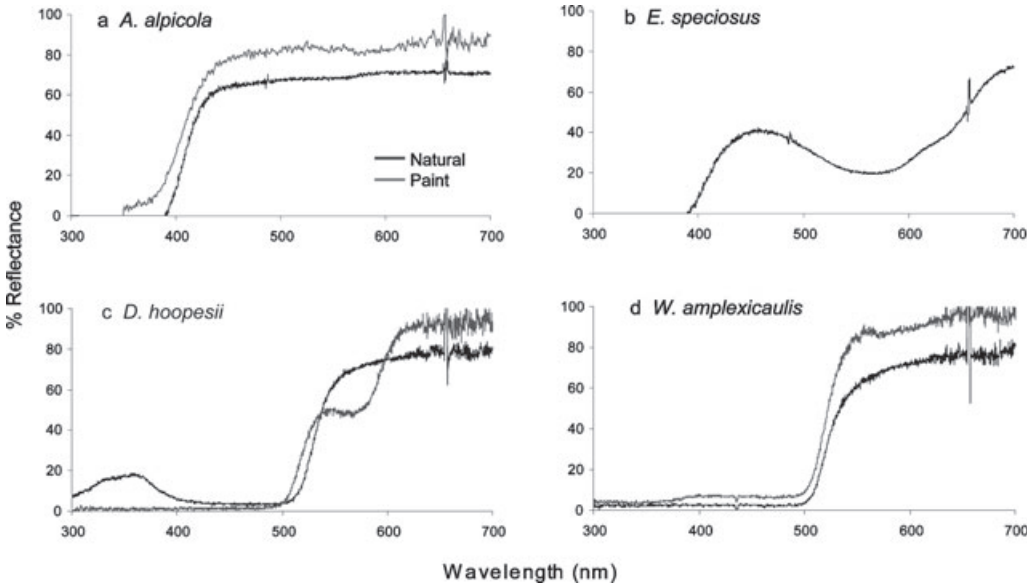


Fig. 1. Average reflectance spectra of unpainted and painted (a) *Achillea alpicola*, (b) *Erigeron speciosus*, (c) *Dugaldia hoopesii*, and (d) *Wyethia amplexicaulis* flowers.

same plants, so that except for newly emerged individuals the butterflies were likely to have experience with these flowers. Since all the butterflies studied have only one brood per year, at least in the study area, as the season progressed the contribution of learned behaviours increased relative to innate behaviours. Each array contained 48, 10–12-cm long, plastic vases (flower picks) spaced every 20 cm and distributed in six by eight rows. Twenty-four flowers from each of two flower types or species were randomly assigned to these vases. Two species pairs were employed, either whole heads of the yellow *W. amplexicaulis* and the orange *D. hoopesii*, or whole flower heads of the purple/blue *E. speciosus* and whole or portions of the white *A. alpicola* floret clusters as units in the arrays. We will refer to these clusters of florets as 'flowers'. All observations in all experiments were made between 8.30 and 17.00 hours.

Flower preference was assessed by treating each foraging bout by an individual butterfly as a replicate, and comparing the proportion of visits butterflies made to each of the flower species with the null expectation of one half, using a one sample *t*-test. Although an arcsin square root transformation is commonly used on proportion data, this transformation did not improve normality of the residuals. We conducted all analyses on the raw proportions. Cases with fewer than five foraging bouts were not analysed. During all observation periods multiple individuals from each butterfly species were observed at the same time visiting the arrays or on flowers in the surrounding area. It is possible the same individuals were observed multiple times, so that inferences are restricted to the observation periods, but all experiments were performed at several different sites and times, minimising the potential for pseudoreplication with respect to generalising across a butterfly species in this region.

To test for flower constancy we recorded the particular sequence of visits in each array and calculated the frequencies of all four possible flight transitions among the two flower types. Two different measures of constancy were calculated. The first was Bateman's (1951) constancy index (B_{index})

$$B_{\text{index}} = \frac{\sqrt{(AD)} - \sqrt{(BC)}}{\sqrt{(AD)} + \sqrt{(BC)}} \quad (1)$$

in which A and D represent the frequencies of transitions between flowers of the same type, and B and C represent the frequencies of transitions between different flower types. Bateman's index distinguishes between complete constancy ($B_{\text{index}} = 1$), random transitions ($B_{\text{index}} \cong 0$), and complete inconstancy ($B_{\text{index}} = -1$), in which all transitions are between unlike flowers (Waser, 1986). Bateman's index cannot be calculated when one of the flight transition categories has no entries. To account for this problem and the difficulty in testing for constancy in visiting bouts showing high preference for one flower type, we also used a modified version of Jacobs (1974) constancy index (C_{index}), as proposed by Gegear and Thomson (2004).

$$C_{\text{index}} = (c - e)/(c + e - 2ce) \quad (2)$$

in which c represents the actual proportion of transitions between same flower types (constant transitions) and e is the expected proportion of transitions between same flower types based on the overall frequency of visitation to a particular flower type. If p is the proportion of visits to flower type 1, then $e = p^2 + (1 - p)^2$. C_{index} also distinguishes between complete constancy ($C_{\text{index}} = 1$), random transitions ($C_{\text{index}} \cong 0$), and complete inconstancy ($C_{\text{index}} = -1$). To test the significance of the obtained values, a randomisation test was used, in which the actual C_{index} was compared with a distribution of 200 values generated under the null hypothesis of no constancy. Each null value for C_{index} was obtained by

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generating a sequence of flower visit transitions of length equal to the observed number, but in which each visit was to species one with probability equal to p. A two-tailed probability was calculated as the proportion of times a value generated under the null hypothesis had an absolute value equal to or greater than the value observed.

Spontaneous flower preferences were examined using arrays containing either *W. amplexicaulis*/*D. hoopesii*, or *E. speciosus*/*A. alpicola* (Table 1). Colour preferences were studied using arrays containing only one flower species, manipulated with water-based acrylic paint so that half of the flowers in the array possessed the colour that matched its own natural coloration and the other half of the flowers were painted to match the colour of the other species in its pair ('colour' arrays, Table 1). For example, one array contained only *A. alpicola* flowers, half of them painted to match the natural colour of the species (white), and half of them painted to match the most abundant colour morph of *E. speciosus* (purple/blue). The accuracy of the match was tested with a spectrometer (Fig. 1, for detailed information on 'colour' arrays refer to Table 1). Each pair of colours tested should have been distinguishable; depending on the species tested, butterflies can distinguish monochromatic lights that differ by 1–5 nm (Koshitaka *et al*., 2008) or 20 nm (Sison-Mangus *et al*., 2008). Water-based acrylic paints have been used previously in studying insect visitation (Waser & Price, 1983; Campbell *et al*., 2010) and do not damage flowers. Control experiments showed that visitation by butterflies is unaffected by whether flowers are painted their original colour or unmanipulated (Document S1).

Painting all flowers in an array the same colour created arrays that erased the contribution of colour to flower preferences. Any preference seen in such an array could be due to differences in floral morphology and size, floral nectar, and/or floral scents. For simplicity, we refer to these arrays hereafter as 'morphology' arrays, recognising that other cues could be involved. An example of such an array would be an array containing all white painted flowers, half of *E. speciosus* and half of *A. alpicola* (Table 1). Morphology arrays of *A. alpicola* and *E. speciosus* flowers (one all white and one all purple/blue) were set approximately 4 m apart, and observed simultaneously. These simultaneous observations allowed us to extract additional information by comparing the number of foraging bouts to both arrays on each of the 4 days using a paired two-sample t-test on log-transformed data.

The size of the floral display can influence flower visitation, potentially explaining any observed 'morphology' preferences. To examine the influence of size, the size of the flower unit used was manipulated in different ways for each flower species, depending on its natural morphology and size. In 'size' arrays consisting of *D. hoopesii*, the ray petals of 24 flowers were cut to half their original length, keeping the remaining 24 intact. Flowers of *A. alpicola* were manipulated by plucking florets until 24 of them were half the size of the other 24 flower clusters. Arrays of *E. speciosus* were manipulated by presenting either single flower heads, or groups of three in a single flower pick. In this case flower preference was assessed by comparing the proportion of visits made by individual butterflies to 'large' (multiple flower heads per flower pick) versus 'small' (single flower head per flower pick) flowers (Table 1).

Flower preferences could also result from attraction to non-visual signals such as association with reward that could cause any of the observed 'morphology' preferences. We measured the standing crop of nectar and its sugar concentration in all four plant species studied, finding that the nectar volume and concentration of *A. alpicola* flower clusters and *E. speciosus* flower heads did not differ. The same result was found for entire flower heads of the *W. amplexicaulis*/*D. hoopesii* species pair (Document S2).

Results

Spontaneous preferences

All five butterfly species showed spontaneous preferences, as they visited one flower species significantly more than the other when foraging for nectar at one or more of the arrays of unmanipulated flowers (Fig. 2). Furthermore, all four butterfly

Table 1. Summary of flower preference array experiments.

Array	Date	Butterflies	Plants	No. of visits (bouts)	Observation hours
Natural	13 July to 7 August 2006	*L. heteronea* and *L. rubidus*	*A. alpicola*/*E. speciosus*	223 (124)	57
Natural	11 July to 13 July 2007	All but *L. rubidus*	*D. hoopesii*/*W. amplexicaulis*	619 (313)	16
Colour	17 July to 22 July 2006	*L. heteronea*	*A. alpicola* blue/white	154 (36)	7
Colour	17 July to 22 July 2006	*L. heteronea*	*E. speciosus* blue/white	36 (30)	8
Colour	25 July to 27 July 2007	All but *L. rubidus*	*D. hoopesii*, yellow/orange	574 (129)	4.5
Colour	25 July to 27 July 2007	All but *L. rubidus*	*W. amplexicaulis*, yellow/orange	378 (94)	7.5
Morph	1 August to 10 August 2007	*L. heteronea*	*A. alpicola*/*E. speciosus*, all white	143 (67)	15.5
Morph	1 August to 10 August 2007	*L. heteronea*	*A. alpicola*/*E. speciosus*, all blue	13 (7)	13.5
Morph	18 July to 2 August 2007	All but *L. rubidus*	*D. hoopesii*/*W. amplexicaulis*, all yellow	196 (104)	18.5
Morph	31 July 2007	All but *L. rubidus*	*D. hoopesii*/*W. amplexicaulis*, all orange	428 (106)	4
Size	12 August to 22 August 2008	All but *L. rubidus*	*E. speciosus*, two sizes	372 (180)	12.5
Size	13 August to 22 August 2008	All but *L. rubidus*	*A. alpicola*, two sizes	209 (157)	11
Size	14 August to 15 August 2008	All but *L. rubidus*	*D. hoopesii*, two sizes	213 (39)	5
				3558 (1386)	**180**

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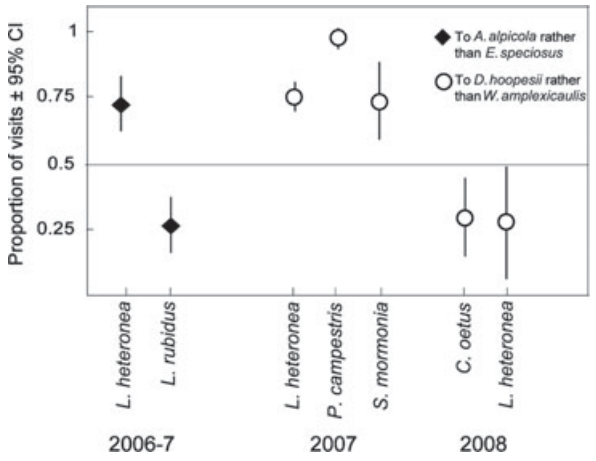


Fig. 2. Spontaneous preferences in arrays of unpainted flowers. Displayed are proportions of visits ±95% CI. Different species of butterflies are arranged along the abscissa.

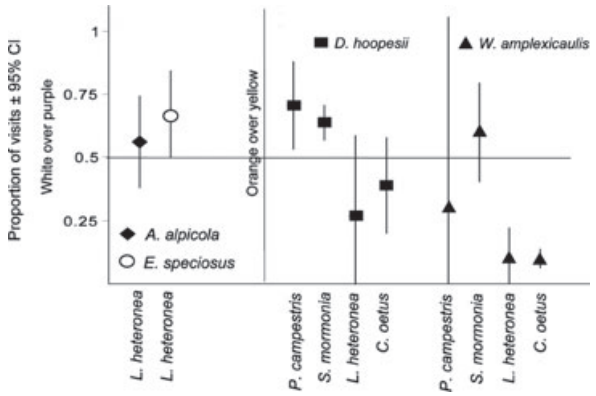


Fig. 3. Spontaneous colour preferences. Arrays were composed of flowers from one species, half of them painted to match its original colour, and half to resemble a second flower species. Displayed are proportions of visits ±95% CI.

species tested on 'colour' arrays (*Lycaena heteronea*, *Speyeria mormonia*, *Cercyonis oetus*, and *Phyciodes campestris*) responded to manipulations of flower colour, although some colour preferences depended on the context of other traits (Fig. 3). Although some sort of response to colour was observed in all of these species, the individual butterfly species differed in colour preference and in the relative importance of preference based on other floral traits. For that reason, we describe the details of the preference results separately for each butterfly species, but with data from all types of experimental arrays together.

Lycaena heteronea

At natural arrays of unpainted flowers, *L. heteronea* preferred to visit *A. alpicola* over *E. speciosus* ($t_{67} = 4.51$, $P < 0.0001$, Fig. 2). In colour preference arrays, *L. heteronea* preferred white over purple *E. speciosus* ($t_{29} = 2.05$, $P < 0.05$, Fig. 3) and while there were no statistically significant preferences for white or purple *A. alpicola* ($t_{21} = 0.72$, $P = 0.24$), on average *L. heteronea* over-visited white flowers (average proportion of white flowers visited = 0.56, Fig. 3). These results suggest that the preference of *L. heteronea* for the normally white-flowered *A. alpicola* is based, at least in part, on a preference for white over purple.

In the 2007 season, *L. heteronea* preferred the orange flowers of *D. hoopesii* over the yellow *W. amplexicaulis*, whereas this preference was reversed in 2008 ($t_{187} = 10.27$, $P < 0.0001$; $t_{19} = 2.27$, $P < 0.05$ respectively, Fig. 2). In terms of colour, *L. heteronea* preferred yellow over orange *W. amplexicaulis*, but showed no colour preferences when choosing between orange and yellow *D. hoopesii* ($t_{24} = 6.93$, $P < 0.0001$; $t_8 = 1.69$, $P < 0.07$ respectively, Fig. 3). In morphology arrays, when all offered flowers were white, *L. heteronea* preferred *A. alpicola* over *E. speciosus* ($t_{66} = 2.58$, $P < 0.05$, Fig. 4), suggesting that a preference for other flower traits exists in addition to one for colour. The flower species visited the most switched from *A. alpicola* to *E. speciosus* when all flowers were painted purple/blue instead of white, indicating that the preference for 'morphological' traits depends upon the context of flower colour. The fact that the purple array had a total of only seven visitation bouts (contrasted with the white array, which was observed simultaneously and visited by 67 individuals; two sample paired t-test: $t_3 = 7.04$, $P < 0.01$) suggests that the preference for the colour white is stronger overall than these preferences for other flower characteristics (Fig. 4).

In *D. hoopesii*/*W. amplexicaulis* morphology arrays, *L. heteronea* had a strong and consistent preference for *W. amplexicaulis* over *D. hoopesii* irrespective of the colour used for the array (orange array: $t_{16} = 3.20$, $P < 0.006$; yellow array 2007: $t_{11} = 2.97$, $P < 0.05$; yellow array 2008: $t_{33} = 1.76$, $P < 0.05$, Fig. 4).

In size arrays, *L. heteronea* preferred larger versus smaller flowers of *A. alpicola* and *E. speciosus* ($t_{101} = 8.72$, $P < 0.0001$; $t_{38} = 3.01$, $P < 0.05$, respectively, Fig. 5). We recorded no visits from *L. heteronea* to *D. hoopesii* size arrays.

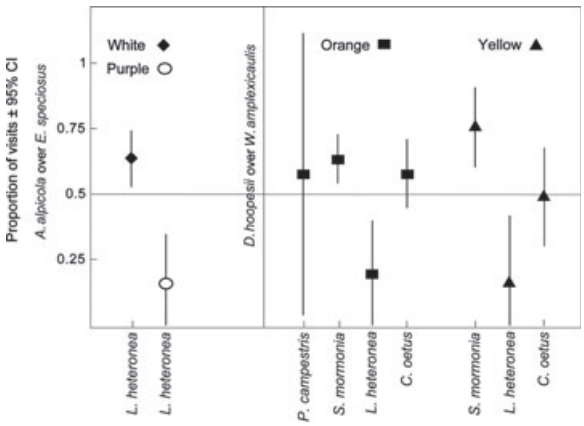


Fig. 4. Spontaneous morphology preferences. Arrays contained all same-coloured flowers from two different species. Displayed are proportions of visits ±95% CI.

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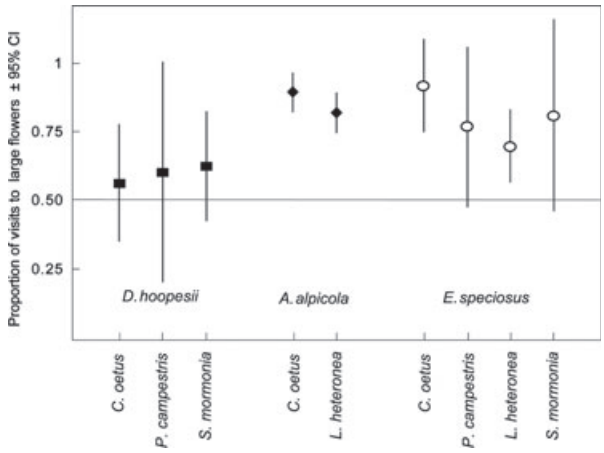


Fig. 5. Spontaneous display size preferences. Arrays contained flowers from one species only. Half of the flower picks presented displays of double the size of the remaining picks. 'Large' *Dugaldia hoopesii*'s flower heads had ray flowers left uncut, whereas 'small' flowers had their ray flowers cut in half. 'Large' *Achillea alpicola* flower clusters were double the diameter of 'small' clusters. 'Large' *Erigeron speciosus* displays included three individual flower heads whereas 'small' displays included only one. Displayed are proportions of visits ±95% CI.

These arrays were observed on the same dates as size arrays of *A. alpicola* and *E. speciosus*, but at a different site, where *L. heteronea* had declined in abundance.

Lycaena rubidus

In contrast to *L. heteronea*, its congener *L. rubidus* preferred to visit *E. speciosus* over *A. alpicola* in both 2006 and 2007 (combined data for both years: average proportion of visits to *A.alpicola* $= 0.26$, $t_{52} = 4.64$, $P < 0.0001$, Fig. 2). Not enough visits to the painted arrays (total of 20 visits to both colour arrays in two seasons) were observed to determine which particular visual cue is driving feeding decisions for this butterfly species.

Speyeria mormonia

Speyeria mormonia preferred the orange flowers of *D. hoopesii* over the yellow *W. amplexicaulis* ($t_{32} = 3.27$, $P < 0.01$, Fig. 2). It preferred orange over yellow *D. hoopesii* but showed no colour preferences when choosing between orange and yellow *W. amplexicaulis* ($t_{79} = 4.07$, $P < 0.0001$; $t_{20} = 1.07$, $P < 0.15$, Fig. 3). Thus, the colour preferences of *S. mormonia* depend on other flower traits, as was the case with *L. heteronea.*

In morphology arrays, *S. mormonia* preferred *D. hoopesii* over *W. amplexicaulis* irrespective of colour (orange array: $t_{59} = 3.00$, $P < 0.005$; yellow array: $t_{25} = 3.52$, $P < 0.005$, Fig. 4). In contrast to *L. heteronea*, *S. mormonia* showed no detectable flower size preferences (*D. hoopesii*: $t_{15} = 1.33$, $P = 0.20$; E. speciosus: $t_6 = 2.17$, $P = 0.07$, Fig. 5). No visits were recorded from *S. mormonia* to *A. alpicola* size arrays. Since these arrays were observed at the same site and dates as size arrays of *E. speciosus*, the lack of visits to *A. alpicola* was not due to a reduced abundance of *S. mormonia*, but instead to its avoidance of *A. alpicola*.

Cercyonis oetus

Visits from *C. oetus* to the natural arrays were observed only in 2008. In 2007 the natural arrays were set-up earlier in the season, before *C. oetus* emerged. This butterfly species preferred *W. amplexicaulis* over *D. hoopesii* ($t_{34} = 2.87$, $P < 0.01$, Fig. 2). In terms of colour, *C. oetus* preferred yellow over orange *W. amplexicaulis* but showed no colour preferences when choosing between orange and yellow *D. hoopesii* ($t_{43} = 21.44$, $P < 0.0001$; $t_{18} = 1.24$, $P < 0.12$, respectively, Fig. 3), thus showing a similar pattern of context-dependent colour preferences as the previous two butterfly species.

In contrast with the butterflies described above, *C. oetus* showed no preferences based on 'morphology' (all flowers orange: $t_{23} = 1.24$, $P < 0.23$; all flowers yellow: $t_{18} = 0.12$, $P = 0.46$, Fig. 4). In size arrays, *C. oetus* preferred larger versus smaller flowers of *A. alpicola* and *E. speciosus* ($t_{54} = 11.34$, $P < 0.0001$; $t_5 = 6.40$, $P < 0.002$, respectively, Fig. 5), but showed no size preferences in *D. hoopesii* arrays ($t_{14} = 0.64$, $P = 0.53$, Fig. 5).

Phyciodes campestris

Phyciodes campestris preferred *D. hoopesii* over *W. amplexicaulis* ($t_{35} = 28.95$, $P < 0.0001$, Fig. 2). It preferred orange over yellow *D. hoopesii* ($t_{20} = 2.50$, $P < 0.05$). Insufficient data were obtained to test for colour preference when choosing between orange and yellow *W. amplexicaulis*.

In common with *Cercyonis oetus* but not the other butterfly species, *P. campestris* had no detectable morphology preferences. When all flowers presented were painted orange, visits to *D. hoopesii* and *W. amplexicaulis* were not significantly different ($t_4 = 0.39$, $P = 0.36$, Fig. 4). No visits to yellow morphology arrays were recorded in either 2007 or 2008. In common with *Speyeria mormonia* but not the other butterfly species, no flower size preferences were found in *P. campestris* (*D. hoopesii*: $t_5 = 0.66$, $P = 0.54$; *E. speciosus*: $t_5 = 2.34$, $P = 0.07$, Fig. 5). Also as in *S. mormonia*, we recorded no visits from *P. campestris* to *A. alpicola* size arrays, despite their proximity (in place and time) to *E. speciosus* arrays, suggesting that *P. campestris* is not attracted to *A. alpicola* flowers.

Flower constancy

During the 2006–2008 field seasons we recorded a total of 2111 flight transitions among flowers in our natural, colour, morphology and size arrays. Despite the fact that different butterfly species showed different colour and morphological preferences, none of them displayed a flower constant behaviour while foraging for nectar in any of the arrays employed (Document S3). None of the constancy indexes departed significantly from zero (Document S3). Point estimates for C_{index}

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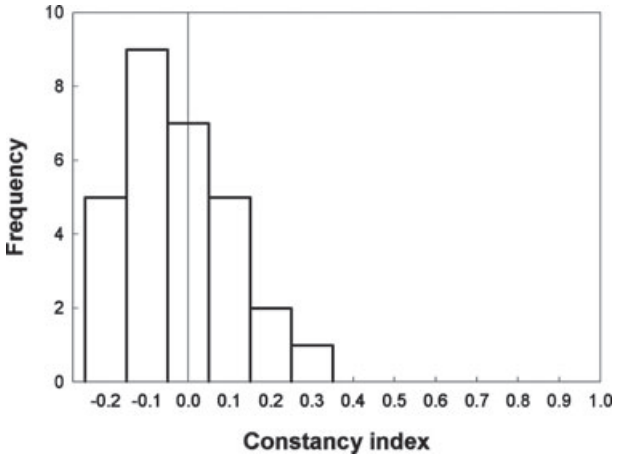


Fig. 6. Distribution of estimates for the flower constancy index C_{index}. The zero line indicates the expectation under the null hypothesis of no constancy. The frequency plotted is the number of combinations of butterfly species and experiment (listed in Document S3) yielding a point estimate for C_{index} within a bin size of 0.1. No individual value for C_{index} differed significantly from zero (all $P > 0.05$).

ranged between −0.22 and 0.33, with a median across all experiments of −0.05 (Fig. 6). In the absence of preference, a value for $C_{index} > 0.28$ would have fallen above the upper 2.5% tail under the null hypothesis of no constancy given a sample size of 50 flower transitions. In our case, 10 out of 29 cases had sample sizes above 50 and expected proportion of visits to like flowers between 0.25 and 0.75, so even when power was reasonably high and preferences were mild, no constancy was detected. Since many foraging bouts were very short, we repeated this analysis using only foraging bouts of 10 or more visits. Similar results were obtained, with the constancy index ranging from −0.36 to 0.02, again with no value significantly different from zero. Bateman's indexes also show transitions indistinguishable from random under a null hypothesis of no constancy (Document S3). No constancy was detected even for experiments with sample sizes as large as 361 transitions. In sum, the chance of visiting a particular flower type never depended significantly on the type previously visited.

Discussion

Preferences

All four butterfly species *Lycaena heteronea*, *Speyeria mormonia*, *Cercyonis oetus*, and *Phyciodes campestris* showed spontaneous preferences between flower species. Moreover, all of these species showed some visitation response to manipulation of flower colour alone, suggesting the potential to exert selection on that trait. Although butterflies were the main visitors of the plant species investigated here, additional information such as the relative effectiveness of each butterfly at pollinating these flowers would be needed to determine how these visitation patterns translate into fitness effects.

Although all of the butterfly species showed preferences while foraging for nectar, they differed in the way they responded to specific visual cues. Both *P. campestris* and *C. oetus* exhibited colour, but no morphology/rewards preferences under these field conditions. These two species seemed to be choosing familiar combinations of visual cues. For example *P. campestris*, which preferred the naturally orange flowers of *D. hoopesii*, significantly over-visited orange over yellow *D. hoopesii* as expected, but when exposed to an array consisting of only *W. amplexicaulis* flowers (whose natural colour is yellow), the trend, although not significant, was in the opposite direction. Even though *C. oetus* did not display significant morphology/rewards preferences, it behaved similarly, over-visiting *D. hoopesii* when all flowers were painted orange, and *W. amplexicaulis* when all flowers were painted yellow. *Speyeria mormonia* strongly preferred flowers from *D. hoopesii*, both because of a preference for the colour orange, and for other flower characteristics.

The phenotypic manipulation of flower colour allowed us to explore the nature of the association between this and other floral traits. These experiments provide one of the few cases demonstrating that pollinators respond to flower colour under field conditions through experimental manipulation of the trait (Rausher, 2008). Colour preferences would not exist without true colour vision, which has been found in all Lepidopteran species examined to date, including hawkmoths (Kelber & Pfaff, 1997), papilionid (Weiss, 1997; Kinoshita *et al*., 1999), pierid (Scherer & Kolb, 1987), and nymphalid (Swihart, 1971) butterflies. Butterfly eyes possess visual pigments of peak sensitivities to wavelengths within the visible light spectrum falling under one of the following categories: ultraviolet (UV, 300–400 nm), blue (B, 400–500 nm) and long wavelength (LW, 500–600 nm) (Briscoe, 2008), with colour vision arising from the interplay between visual pigments of different spectral sensitivities. The remarkable diversity in the number, distribution and specific properties of visual pigments among butterflies (Briscoe, 2008) suggests correspondingly diverse ways of perceiving colours that could partially underlie the distinct behavioural responses observed in this study.

The use of both colour arrays and 'morphology' arrays allows consideration of whether butterflies respond to the two kinds of traits in the way that is predicted from responses to each alone. The behaviour displayed by *L. heteronea* provides strong evidence that combinations of traits can be critical to butterfly visitation. In simultaneously observed white and purple morphology arrays of *A. alpicola* and *E. speciosus*, it significantly preferred *A. alpicola* over *E. speciosus* when both were painted white, but made the reverse choice when both were painted purple. Its behavioural response to one trait thus depended upon the context of other floral traits.

The 'morphology arrays' we used did not directly distinguish between responses to rewards or flower size versus flower morphology *per se*. However, the flower units of *A. alpicola* and *E. speciosus* did not differ in either nectar volume or concentration, nor did the flower units of *D. hoopesii* and *W. amplexicaulis*. Thus we can rule out the roles of these traits in producing the apparent morphology preferences. On the other hand, larger flower display sizes were generally chosen

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over smaller ones, though only *C. oetus* and *L. heteronea* preferences were significant. These size preferences could have confounded our results from arrays consisting of combinations of *D. hoopesii* and *W. amplexicaulis*, since the latter's flower heads are generally larger than *D. hoopesii*'s (N. Pohl, pers. obs.). However, no butterfly species showed size preferences in *D. hoopesii* arrays; moreover, three of the butterfly species showed a consistent preference for the smaller-flowered *D. hoopesii*, which clearly cannot be explained by a preference for large size. With the present information we cannot rule out the role of size as a trait partly responsible for the preference of *C. oetus* for *W. amplexicaulis* flowers. Larger flower and flower display sizes preferences have been previously described in Lepidoptera (Vaughton & Ramsey, 1998; Arroyo *et al*., 2007).

Our study included one pair of congeneric butterflies, making it of interest to compare their behaviour. *Lycaena rubidus* shares the geographical range and habitats of the closely related *L. heteronea* (Glassberg, 2001; Pratt & Wright, 2002), and possesses the same combination of four visual pigments in the ventral half of the compound eye (Sison-Mangus *et al*., 2006). Flying over two-dimensional landscapes such as the mountain meadows where we conducted this study, these butterflies are presumably detecting flowers with these ventral ommatidia, implying that in principle the two species can detect the same flower colours. In this light, it is interesting that *L. heteronea* and *L. rubidus* preferred different flower species (and presumably colours). Differences in spontaneous flower colour preferences can arise from a variety of mechanisms. First, two butterfly species may prefer different flower colours due to innate or learned preferences. Butterfly species may differ in their innate colour preferences not only by possessing a different set of visual pigments or exhibiting variation in their distribution in the compound eye, but also by differing at higher levels of information processing (as suggested by Raine & Chittka, 2007b). For lepidopterans, differences in innate colour preferences have been found among genera, species, and even between sexes within a species (Goulson & Cory, 1993; Kelber & Pfaff, 1997; Kinoshita *et al*., 1999). Innate colour preferences of lepidopterans can often be readily altered by learning, as shown in hawkmoths (Kelber & Pfaff, 1997), some nymphalids (Weiss, 1995), the papilionid *Battus philenor* (Weiss, 1997), and two species of *Pieris* (Lewis & Lipani, 1990). Learning to prefer different flower colours could be beneficial if two butterfly species are competing for resources. For example, the difference in spontaneous flower preferences shown by the two *Lycaena* species could be due not to dissimilar perceptual capabilities but to ecological factors such as aggressive competition for floral resources, as was observed in two Brazilian *Heliconius* species (Rocha & Duarte, 2001). Although the flower units of *A. alpicola* and *E. speciosus* did not differ in either nectar volume or concentration, it is also possible that the two *Lycaena* species could respond differently to other floral characteristics such as amino acid composition of the nectar and/or flower scent. For example, *Inachis io* butterflies prefer solutions that contain amino acids over otherwise identical solutions lacking them (Erhardt & Rusterholz, 1998). Butterflies can also respond to and learn new flower scents, although, for experienced butterflies, scent is sometimes of secondary importance compared with colour (Ômura & Honda, 2005).

In 2007, the behaviour displayed by *L. heteronea* was initially puzzling. It chose flowers based on both colour and morphology/rewards, but its preferences were not coherent. It preferred the normally orange *D. hoopesii*, but when colour and morphology/rewards were decoupled, it seemed to prefer the colour yellow and the morphology/rewards of *W. amplexicaulis.* One possible explanation is that the flower preferences of *L. heteronea* shifted throughout the season. The arrays with unpainted flowers of both species were utilised only at the beginning of the field season, followed by the painted arrays, and therefore *L. heteronea* butterflies could have learnt to avoid its originally preferred species, switching to a diet consisting primarily of *W. amplexicaulis* flowers, and concurrently switching its colour preference to yellow and its morphology preference to *W. amplexicaulis*. Since the resources of the two species were not significantly different in sugar concentration or quantity of nectar, such switching behaviour could be beneficial for *L. heteronea* only if other nectar qualities such as amino acids differed (see Erhardt & Rusterholz, 1998) or if the demand for *W. amplexicaulis* was lower than for the heavily used flowers of *D. hoopesii*. In this scenario, if *W. amplexicaulis* flowers possess characteristics unattractive to some butterfly species other than *L. heteronea* (e.g. a particular scent, set of available amino acids), visits to *D. hoopesii* could offer less and less reward compared with *W. amplexicaulis* as the season progresses.

Constancy

The striking absence of flower constancy found in this study contrasts with the few reports suggesting constancy in butterflies (Lewis, 1986, 1989; Goulson & Cory, 1993; Goulson *et al*., 1997a,b; but see Kandori & Ohsaki, 1996). However, none of those studies used experimental manipulations of real flowers to approach the question of constancy under field conditions, but instead either utilised natural or artificial flowers in artificial settings, or followed free flying butterflies recording all flowers visited in a certain area. The drawback of the latter approach is that the per cent of conspecific transitions is a function not just of constancy, as in an experiment with randomly arranged flowers, but also of the natural spatial arrangement of the flowers. Furthermore, several of these studies did not test constancy *per se* but measured handling and/or searching times, behaviours that influence the advantage of constancy to the visitor. Flower constancy has been described for several groups of pollinating insects including honeybees (Grant, 1950), bumblebees (Wilson & Stine, 1996; Gegear & Laverty, 2004; Raine & Chittka, 2005), stingless bees (Slaa *et al*., 1998), solitary bees (Gross, 1992), beetles (De Los Mozos Pascual & Domingo, 1991; Mico & Galante, 2005), and flies (Goulson & Wright, 1998). Their degree of flower constancy varies, depending on factors such as the colour of the flowers presented (Hill *et al*., 2001), nectar volume, and inter-flower distance (Gegear & Thomson, 2004; Raine

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& Chittka, 2007a). Bumblebees can average up to 0.8 for the constancy index employed here (Gegear & Thomson, 2004), contrasting sharply with our results for butterflies (Fig. 6). In our study, aggression of male butterflies chasing off other foragers kept most foraging bouts short, which could reduce the tendency to develop constancy, but even for longer bouts we saw no evidence of constancy under these field conditions. Due to the intrinsic cognitive constraints in learning and remembering different flower types, as the flower types differ more in various traits, visit behaviour is expected to become more constant (Waser, 1986; Chittka *et al*., 1999). All of the experiments described in this study employed flowers in the Asteraceae, which share similar inflorescence architecture and therefore may have been too alike for some butterfly species to detect their differences and develop constancy in 'morphology' arrays. This could be the case for *C. oetus* and *P. campestris*, species that showed no morphological preferences, but not for *L. heteronea* and *S. mormonia*, whose clear morphology preferences indicate that, regardless of this common inflorescence architecture, these butterflies could distinguish between the flowers used in the arrays.

Flower constancy can benefit plant reproduction by increasing assortative pollination between conspecifics, whereas the absence of constancy could be disadvantageous for plant species that rely mostly on butterflies for pollen transfer. Nonetheless, successful butterfly pollination has been unequivocally shown on many occasions (Levin, 1968; Schmitt, 1980; Arroyo *et al*., 1982; Courtney *et al*., 1982; Bloch & Erhardt, 2008). Flower inconstancy can decrease plant reproductive success by lowering visitation by more constant visitors (Feinsinger, 1987), pollen loss to foreign stigmas (Campbell & Motten, 1985), stigma clogging and interference from foreign pollen (Thomson *et al*., 1981), and production of hybrids (Levin, 1972; Campbell & Aldridge, 2006). These plant fitness costs could potentially be offset by the advantage of having more visitors, regardless of their degree of constancy (Chittka *et al*., 1999). In addition, species that share inconstant pollinators may reduce the costs of inconstant pollinators by placing their pollen loads on different body parts of their visitors (Dressler, 1968; Armbruster & Herzig, 1984). For butterflies, the magnitude of these various fitness costs associated with inconstant foraging are unknown.

Conclusions

In summary, we found that butterflies possess flower preferences in the field based on a variety of floral traits. All four of the butterfly species showed some visitation response to manipulation of flower colour alone, suggesting the potential to exert selection on that trait as has been demonstrated in other pollinator types. Moreover, colour preferences often depended on the context of other floral traits. In contrast with findings in honeybees and bumblebees, the studied butterflies showed no flower constancy in their foraging bouts, at least when confronted with two species from the Asteraceae. This inconstancy has the potential to reduce the effectiveness of butterfly visits, suggesting that future studies should examine the consequences of preference, along with absence of constancy, for selection on floral traits and for the relative effectiveness of butterflies compared with other pollinators.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2011.01271.x

Document S1. Paint control experiments.

Document S2. Nectar rewards.

Document S3. Tests of flower constancy behaviour.

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