

Interspecific and intersexual learning rate differences in four butterfly species

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SUMMARY

Learning plays an important role in food acquisition for a wide range of insects and has been demonstrated to be essential during flower foraging in taxa such as bees, parasitoid wasps, butterflies and moths. However, little attention has been focused on differences in floral cue learning abilities among species and sexes. We examined the associative learning of flower colour with nectar in four butterfly species: *Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*. All butterflies that were trained learned the flower colours associated with food. The flower colour learning rates were significantly higher in *I. leuconoe* and *A. hyperbius* than in *P. rapae* and *L. phlaeas*. Among the four species examined, the larger and longer-lived species exhibited higher learning rates. Furthermore, female butterflies showed a significantly higher learning rate than males. This study provides the first evidence that learning abilities related to floral cues differ among butterfly species. The adaptive significance of superior learning abilities in the larger and longer-lived butterfly species and in females is discussed.

Key words: associative learning, forewing length, innate colour preference, Lepidoptera, lifespan.

INTRODUCTION

The learning abilities of animals are adaptive (e.g. Dukas and Duan, 2000), and a high learning ability increases fitness (Raine and Chittka, 2008). A variety of insects extensively rely on learning for all major life activities, including feeding, predator avoidance, aggregation, social interaction and sexual behaviour (Dukas, 2008). The ability to learn while foraging for flowers has been demonstrated in many insect taxa, such as bees (e.g. Dukas and Real, 1991; Heinrich, 1976; Heinrich, 1979; Laverty and Plowright, 1988; Menzel, 1985; Menzel, 1993), wasps (e.g. Sato and Takasu, 2000; Shafir, 1996; Takasu et al., 2007), hoverflies (Goulson and Wright, 1998), butterflies (e.g. Kandori and Ohsaki, 1996; Kandori and Ohsaki, 1998; Kinoshita et al., 1999; Lewis, 1986; Lewis, 1989; Swihart and Swihart, 1970) and moths (e.g. Cunningham et al., 2004; Cunningham et al., 1998; Kelber, 1996; Kelber, 2002). For example, butterflies and moths can rapidly undergo reverse conditioning when rewarding and unrewarding flower colours or odours are exchanged (e.g. Fan and Hansson, 2001; Goulson and Cory, 1993; Kelber, 1996; Weiss, 1997). With increased experience, bees and butterflies can improve their flower handling skills and/or shorten the time required to find a nectar or pollen source in flowers (e.g. Kandori and Ohsaki, 1996; Laverty, 1980; Lewis, 1986; Raine and Chittka, 2007). Moreover, honeybees and hawkmoths are able to remember unrewarding patterns or colours and avoid them (Kelber, 1996; Srinivasan et al., 1994). Together, these studies indicate that flower-visiting insects are generally good learners.

Some studies have demonstrated that flower foraging learning abilities differ among individuals (e.g. Worden et al., 2005) and among colonies (Raine and Chittka, 2008) within a bumblebee species. However, little attention has been focused on differences in learning abilities among insect species. In fact, to our knowledge, only one study has comparatively evaluated this phenomenon and

revealed that a social species of bumblebee, *Bombus bimaculatus*, demonstrated higher learning rates in discriminating rewarding flower colours compared with a solitary species of carpenter bee, *Xylocopa virginica* (Dukas and Real, 1991).

Like bees, butterflies may also show differences in learning abilities among species. The arguments of Lewis and Lipani (Lewis and Lipani, 1990) lead to the prediction that longer-lived and larger butterfly species may exhibit higher learning abilities than do shorter-lived and smaller butterfly species. However, this hypothesis has not yet been tested.

Intersexual differences in floral foraging learning have only been detected in bumblebees (Church et al., 2001), moths (Hartlieb et al., 1999) and butterflies (Kroutov et al., 1999). In the butterfly *Agraulis vanillae*, Kroutov et al. reported that more females than males were conditioned to chemical stimuli associated with food (Kroutov et al., 1999). However, sexual differences in flower colour learning in butterflies have not been investigated.

In this study, we examined the associative learning of flower colour with nectar in four butterfly species: *Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas* (Lepidoptera: Papilionoidea). We specifically addressed two questions: (1) are there differences in associative learning abilities, such as the learning rate of flower colour, among butterfly species or between sexes? (2) Are interspecific differences in learning abilities correlated with body size or lifespan? To our knowledge, this is the first empirical investigation of interspecific and intersexual differences in flower colour learning abilities among butterfly species.

MATERIALS AND METHODS

Experimental preparation

The four experimental species of butterflies are considered generalist flower visitors. They were originally collected and reared as described below.

***Idea leuconoe* (Butler) (Danainidae)**

We used laboratory-reared individuals from the Itami City Museum of Insects, Itami, Osaka, Japan. Butterflies were originally collected in Ishikawa, Uruma, Okinawa, Japan. One to three larvae were reared in a 500 ml or 860 ml transparent plastic cup on fresh leaves of *Parsonia alboflavescens* (Dennstedt) Mabberley at 22–25°C and 15 h:9 h L:D in an incubation room.

***Argyreus hyperbius hyperbius* (Linnaeus) (Nymphalidae)**

Adult females and larvae were obtained near the Nara campus of Kinki University, Nakamachi, Nara, Japan, from July to August 2004. Adult females were then allowed to oviposit eggs. About 10 larvae were reared together on fresh leaves of several *Viola* species in 450 ml transparent plastic cups at room temperature (range of daily mean: 24–30°C) and natural daylength (range: 14 h:10 h L:D to 15.5 h:8.5 h L:D) within the laboratory.

***Pieris rapae crucivora* (Boisduval) (Pieridae)**

Adult females and larvae were collected near the Nara campus of Kinki University from September to October 2004. Adult females oviposited eggs, and the larvae were reared on fresh leaves of *Brassica oleracea* L. and *Raphanus sativus* L. in 450 ml transparent plastic cups (10 larvae per cup) at room temperature (14–29°C) and natural daylength (12 h:12 h L:D to 14 h:10 h L:D) within the laboratory.

***Lycaena phlaeas daimio* (Matsumura) (Lycaenidae)**

Adult females were obtained near the Nara campus of Kinki University from March to April 2004. Adult females were allowed to oviposit eggs. About 10 larvae were reared together on fresh leaves of *Rumex acetosa* L. and *Rumex japonicus* Houtt in 450 ml transparent plastic cups at room temperature (10–20°C) and natural daylength (13 h:11 h L:D to 14.5 h:9.5 h L:D) within the laboratory.

We assumed that seasonal changes in rearing conditions would not affect the species-specific learning abilities of butterflies. Thus, the rearing conditions of *I. leuconoe* were assumed to be within the range of seasonal changes in rearing conditions of the other three butterfly species. After eclosion, each adult was numbered on the hindwings with an oil-soluble marker and kept without food at 15°C and 12 h:12 h L:D until the experiments were started. All butterflies were of similar age (1–3 days old) at the start of the experiments.

Experimental location

We examined the foraging behaviour of butterflies in either a mesh field cage (1.9 m × 1.9 m × 1.9 m) under natural conditions (range of daily mean temperature during the experiment: 12–28°C) or in an indoor incubation room (2.7 m × 1.8 m × 2.0 m) on the Nara campus of Kinki University. In the incubation room, light was provided from the ceiling by 10 fluorescent tubes (Truelite EX-VS, 40 W; ELC, Philadelphia, PA, USA) with emission spectra closely approximating that of sunlight. The room was covered in a black nylon net, except for the ceiling. The room temperature was set at 24°C.

Artificial flowers

Artificial flowers made from discs of coloured paper were used as stimuli (Daiei Training colour 200, Tokyo, Japan). The flower diameters were relative to the species' body size and were 5 cm for *I. leuconoe* and *A. hyperbius*, 4 cm for *P. rapae* and 3 cm for *L. phlaeas*. The nationwide collection of field data suggested that smaller butterfly species utilise smaller flowers and larger butterflies utilise larger flowers. For example, the mean floral diameter of a

frequently visited plant species by the large butterfly *Papilio xuthus* was 5.10 ± 0.81 cm (mean ± s.e.m.; $N=25$) whereas the mean flower diameter was 2.94 ± 0.48 cm ($N=73$) for the medium-sized butterfly *P. rapae* and 2.05 ± 0.27 cm ($N=54$) for the small butterfly *L. phlaeas* (Tanaka, 1982). From these results, we thought it appropriate to use smaller artificial flowers for smaller butterfly species.

Rewarding flowers were made from a disc of coloured paper that had a 5 mm diameter hole at the centre with an attached Eppendorf tube (1.5 ml) containing a 10% sucrose solution. Unrewarding flowers only consisted of a disc of coloured paper with a hole at the centre. All flowers were set on a green coloured plastic circle frame (30 cm in diameter). Flower number and attached colour differed according to the experiment, the part or the session (see fig. below). The plastic frame was raised 60–70 cm above the ground from the centre of the experimental location and exposed to the butterflies. During all behavioural experiments, the frame was turned 90 degrees every 20 min so that flower position would not be a learning factor.

General experimental rules

We defined a 'visit' as a positive response when a butterfly landed and extended its proboscis toward the coloured paper. In all behavioural experiments, 10–40 butterflies of each species or each group within a species (see below) were released at the same time and allowed to visit the artificial flowers. When a butterfly visited any flower for a set number of times (i.e. five times, except during the training session for the second part of experiment 2, in which each butterfly was allowed to visit only once; see below), we temporarily removed that butterfly from the experimental location until the end of the experiment, the part or the session. Recent studies have reported that bumblebees can copy the flower choice of experienced foragers, which is considered social learning (Leadbeater and Chittka, 2005; Worden and Papaj, 2005). However, in insects, social learning has only been demonstrated in the social Hymenoptera. Therefore, conducting experiments with multiple individuals at the same time and place should not affect individual flower choices.

Experiment 1: innate colour preferences among 12 colours

To determine which colours should be used in the colour learning experiment, the innate colour preference was investigated by allowing naïve butterflies to visit 12 artificial flowers coloured red, red–purple, purple, blue, green, yellow–green, yellow, orange, brown, light blue, white and pink. The preference of each butterfly was recorded over five visits. The experiments for *A. hyperbius*, *P. rapae* and *L. phlaeas* were conducted in the field cage on sunny–cloudy days from April to October 2004. The experiment for *I. leuconoe* was conducted in the incubation room from October to November 2005 when it was too cold outside for this species to actively forage.

Experiment 2: colour learning

According to the results from experiment 1, two artificial flower colours were chosen and used for each butterfly species. The first and the second most preferred colours were red and orange for *I. leuconoe*, orange and white for *A. hyperbius*, blue and yellow for *P. rapae*, and yellow and orange for *L. phlaeas*, respectively (see Results). We first tested the innate colour preference between the two colours in naïve butterflies. To do this, we used eight flowers (four flowers for each colour) that we alternatively set without rewards, and each individual butterfly was allowed to visit the flowers five times. The colour that an individual visited three or

more times was defined as the preferred colour by that individual. All individuals were then separated into two groups according to their innate colour preference. This part of the experiment was conducted over a 2–3-day period for each butterfly species. Next, we examined flower colour learning through a training session, followed by a test session. In the training session, butterflies from each of the two colour groups were trained to feed on the innately less-preferred colour, i.e. four flowers of the less-preferred colour were set with rewards, and each individual was allowed to visit a flower, drink rewards and spontaneously leave the flower once. Butterflies that did not spontaneously visit a flower were manually placed on the flower. These individuals usually learned to spontaneously feed on the flower within two days. The test session was similar to the first part of this experiment in that we set eight flowers (four of each of the two colours) without rewards, and the preference of each individual was recorded over five visits. The second part of this experiment was conducted every day for six days. Trainings were conducted in the morning (10:00h–11:30h) and tests were conducted in the afternoon (13:00h–16:00h). We rejected individual butterflies that did not finish the task within a fixed amount of time (i.e. 1.5h for trainings and 3h for tests). Out of all of the individuals that began the experiments, approximately 50–80% of each butterfly species completed all of the tasks (seven tests with six trainings between each test). Only those that finished all tasks were used in the statistical analysis, i.e. 26 and 23, 47 and 49, 47 and 46, and 46 and 43 individual females and males of *I. leuconoe*, *A. hyperbius*, *P. rapae* and *L. phlaeas*, respectively. The number of individuals in the two training colour groups was nearly equal for both sexes in each butterfly species.

Experiment 3: forewing length and lifespan

This experiment was conducted to examine whether differences in learning rates were correlated to body size or lifespan among species. Forewing length of adult butterflies was used as an indicator of body size and was measured within each species. The number of measured individuals was 16 and 15, 18 and 17, 17 and 18, and 19 and 20 for females and males of *I. leuconoe*, *A. hyperbius*, *P. rapae* and *L. phlaeas*, respectively. The lifespan of adult butterflies was measured in the incubation room under controlled conditions (22°C and 14h:10h L:D) in 2004–2005. In the incubation room, each *I. leuconoe* and *A. hyperbius* butterfly was kept within a transparent plastic cylinder (20 cm diameter × 30 cm height) covered with mesh on the top, and each *P. rapae* and *L. phlaeas* was kept within a 450 ml transparent plastic cup. The butterflies were fed at approximately 12:00h noon each day from adult eclosion until death. At each feeding, individuals were allowed to feed from cotton containing a 10% sucrose solution until they spontaneously re-coiled their proboscises. Lifespan was measured in 10 and 11, 12 and 14, 19 and 18, and 10 and 10 individual females and males of *I. leuconoe*, *A. hyperbius*, *P. rapae* and *L. phlaeas*, respectively.

Different individuals were used in each experiment and for the measurements of forewing length and lifespan in experiment 3.

Statistical analysis

To compare learning rates among the four butterfly species and between the two sexes or colours, we used a general non-linear learning model:

$$P = 1 - (1 - P_0) e^{-aN}$$

where P is the proportion of butterfly visits to a rewarding flower colour, a is the learning rate and N is the number of trainings on a rewarding flower colour. P_0 refers to P at $N=0$, or the innate colour

preference. This model represents the general form observed in our learning experiments: the rate of change in learning was initially higher and gradually diminished as the individual trained on more flowers. The proportion of visits to a rewarding flower colour asymptotically approached 1. If P_0 is constant, then a higher a generates a learning curve that approaches 1 more rapidly (Fig. 1A). If a is constant, then a curve with a higher P_0 is identical to a curve with a lower P_0 when the latter slides to the left until it wraps over the former and cuts it off at $N < 0$ (Fig. 1B). Therefore, a is not affected by the height of the starting point (P_0). This learning curve was fitted to seven data points, representing seven tests, for each individual butterfly to estimate P_0 and a . We then used a fixed-effects analysis of variance [ANOVA; general linear model (GLM) with Type III sums of squares] to test for effects on learning rate (log+1-transformed), with species, sex, colour and their pairwise and three-way interactions as independent factors. We also used a fixed-effects ANOVA to test for effects on forewing length (data were not transformed because they met the assumption of heterogeneity; Levene test; $P > 0.05$) and to test for effects on lifespan (log+1-transformed), both of which included species, sex and their pairwise interactions as independent factors. Tukey's honestly significant difference (HSD) tests were used to perform *post hoc* multiple comparisons. To test whether larger or longer-lived species exhibited higher learning rates, an analysis of covariance (ANCOVA) was used that included sex as an independent factor and forewing length or survival period as a covariate. To simplify the model, we did not include flower colour as a factor because flower colour did not affect learning rate in the ANOVA analysis (see Results). In this model, we only used eight data points. Each of these data points represented a mean for each sex of each species

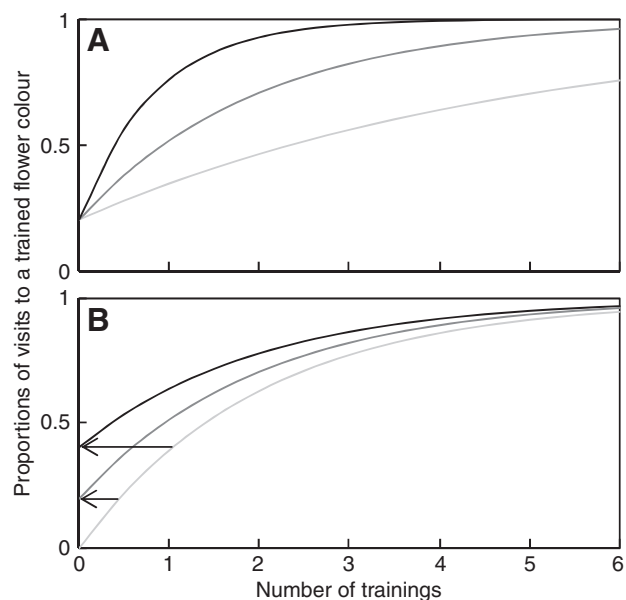


Fig. 1 General properties of learning curves drawn by the equation $P = 1 - (1 - P_0) e^{-aN}$. The curves illustrate how improvement in task performance over time is related to the learning rate a (A) and innate colour preference P_0 (B). Panel A shows learning curves for a fast ($a=1.2$, black line), intermediate ($a=0.5$, grey line) and slow ($a=0.2$, light grey line) learner with a constant height at the starting position ($P_0=0.2$). Panel B shows the curves for an intermediate learner ($a=0.5$) with high ($P_0=0.4$, black line), intermediate ($P_0=0.2$, grey line) and low ($P_0=0$, light grey line) starting positions. Arrows indicate that if a is constant, a curve with a higher P_0 is identical to a curve with a lower P_0 but is shifted to the left.

(i.e. two sexes \times four species). SPSS 14.0 software (SPSS, 2005, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Innate colour preferences

From the 12 possible colours, naïve *I. leuconoe* females and males preferred red (20.0 and 16.0%, respectively) followed by orange (15.6 and 14.0%, respectively) out of a total of 45 and 50 visits, respectively. Naïve *A. hyperbius* females and males preferred orange (15.8 and 17.2%, respectively) followed by white (13.5 and 13.6%, respectively) in a total of 260 and 250 visits, respectively. For *P. rapae*, naïve females preferred yellow (19.2%) followed by blue (18.8%) in a total of 245 visits whereas naïve males preferred blue (20.9%) followed by yellow (17.8%) in a total of 230 visits. Lastly, naïve *L. phlaeas* females and males preferred yellow (36.1 and 36.9%, respectively) followed by orange (29.8 and 30.0%, respectively) in a total of 255 and 260 visits, respectively. In summary, the first and second most preferred colours by naïve

butterflies were red and orange for *I. leuconoe*, orange and white for *A. hyperbius*, and yellow and orange for *L. phlaeas*, respectively. In the case of *P. rapae*, the two most preferred colours were opposite between the sexes. In this species, we defined blue and yellow as the first and second most preferred colour, respectively, based on the mean preference of the two sexes.

Colour learning

Both females and males of the four butterfly species exhibited typical learning curves, i.e. as the number of times an individual was trained on a particular flower colour increased, the rate of selection for that colour increased (Fig. 2). When the two training colour groups were combined, the mean learning rate (log+1-transformed) was always higher for females compared with males within the same species (Fig. 3). When females and males were separately compared among species, the mean learning rate was highest for *I. leuconoe*, followed by *A. hyperbius*, *P. rapae* and lastly by *L. phlaeas* within both females and males (Fig. 3). The ANOVA for learning rate indicated

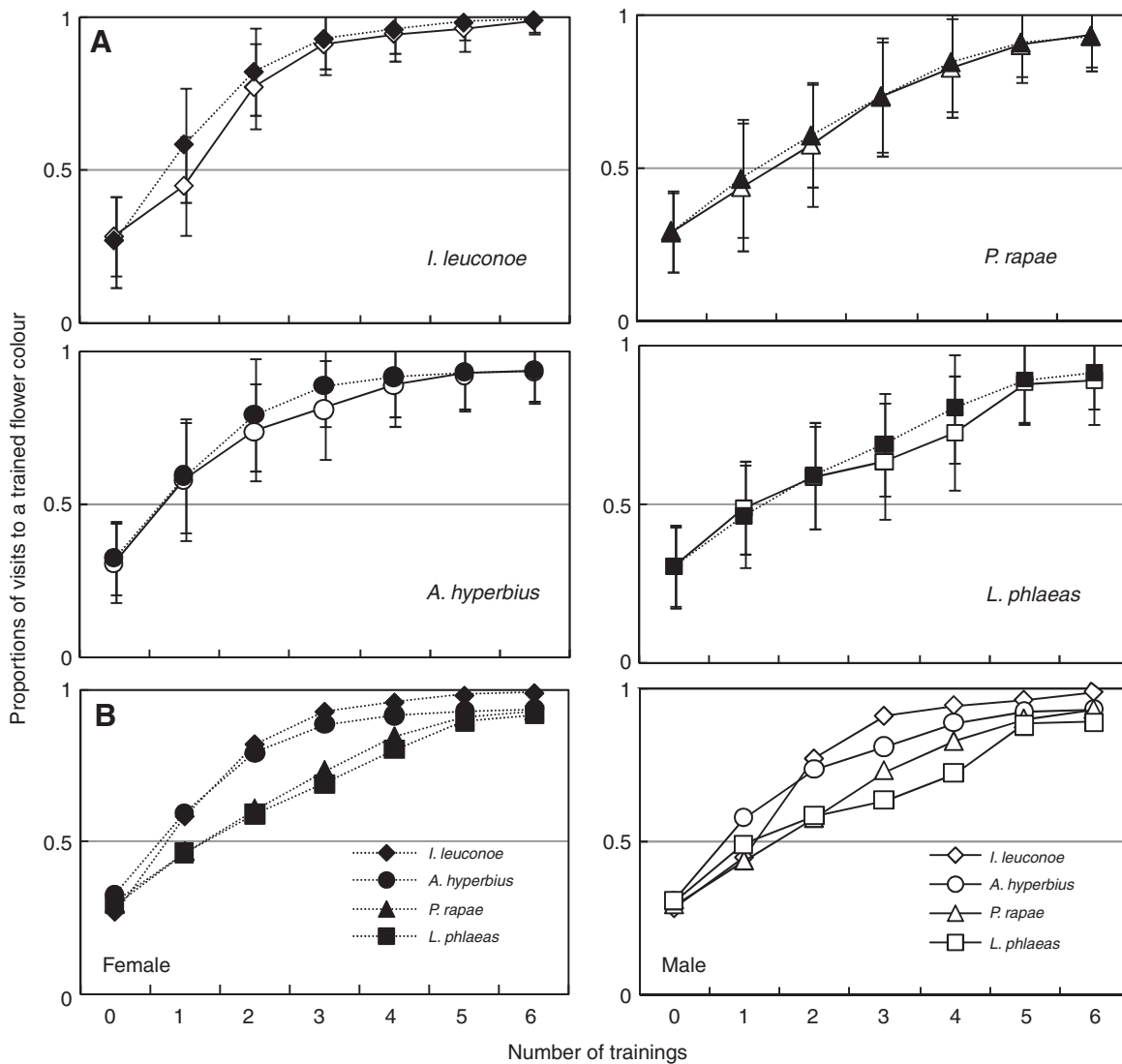


Fig. 2 Effect of training on visits to the trained artificial flower colour for females (closed symbols) and males (open symbols) in the four butterfly species (*Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*). Panel A shows differences between sexes within the same butterfly species. Panel B shows differences among four butterfly species within the same sex. Because flower colour did not affect the learning rate (Table 1), the two training colour groups were combined within each sex for each butterfly species. For the number of individuals, see Materials and methods. Each data point in A represents the mean value \pm s.d. whereas s.d. was eliminated in B.

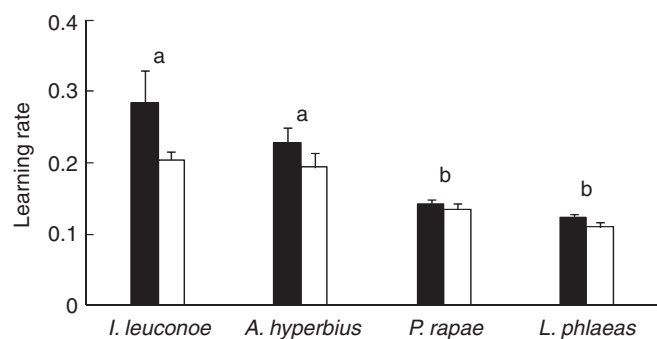


Fig. 3 Learning rate (log+1-transformed) for females (closed bars) and males (open bars) in the four butterfly species (*Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*). Because flower colour did not affect learning rate (see Table 1), the two training colour groups were combined within each sex for each butterfly species. The letters above the bars indicate significant differences among species (Tukey's HSD: $P < 0.05$). For the number of individuals, see Materials and methods. Each bar represents the mean value \pm s.e.m.

that there were significant species and sex effects and that colour and all interactions were not significant (Table 1). *Post hoc* multiple comparisons among species revealed that the learning rates of *I. leuconoe* and *A. hyperbius* were significantly higher than those of *P. rapae* and *L. phlaeas* whereas there were no significant differences between *I. leuconoe* and *A. hyperbius* or between *P. rapae* and *L. phlaeas* (Fig. 3).

Forewing length and lifespan

Mean forewing length was longest in *I. leuconoe*, followed by *A. hyperbius*, *P. rapae* and lastly *L. phlaeas*, for both females and males (Fig. 4A). Our ANOVA analysis of forewing length indicated that the species effect was significant and that sex and their interaction effects were not significant (Table 2A). *Post hoc* multiple comparisons revealed that forewing length was significantly different among species (Fig. 4A). The mean lifespan was also longest for *I. leuconoe*, followed by *A. hyperbius*, *P. rapae* and *L. phlaeas*, within both females and males (Fig. 4B). The ANOVA for lifespan indicated a significant effect of species and non-significant effects of sex and their interaction (Table 2B). *Post hoc* multiple comparisons revealed that lifespan was significantly different among species (Fig. 4B). An ANCOVA revealed that learning rate was

Table 1. ANOVA of learning rate

Source	d.f.	MS	F	P
Species	3	0.260	22.795	0.000
Sex	1	0.085	7.436	0.007
Colour	1	0.019	1.698	0.194
Species \times sex	3	0.017	1.451	0.228
Species \times colour	3	0.011	1.007	0.390
Sex \times colour	1	0.011	0.969	0.326
Species \times sex \times colour	3	0.008	0.727	0.537
Error	311	0.011		

Species refers to the effect of the four butterfly species (*Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*). Sex refers to male and female effects. Colour refers to the effect of the two flower colours that were used in each butterfly's training (the first and the second most preferred colour by naive butterflies for each species). Significant differences are in bold.

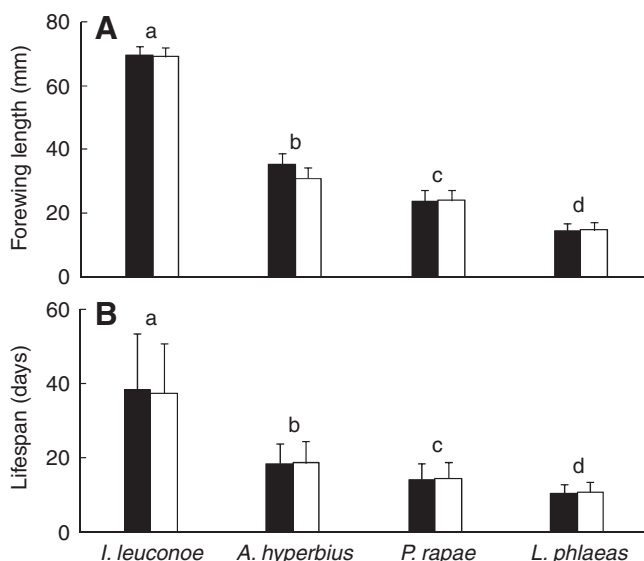


Fig. 4 Forewing length (A) and lifespan (B) for females (closed bars) and males (open bars) of the four butterfly species (*Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*). The letters above the bars in each figure indicate significant differences among species (Tukey's HSD: $P < 0.05$). For the number of individuals, see Materials and methods.

positively correlated with forewing length and lifespan among species (Table 3).

DISCUSSION

Our results showed that innate colour preference differed among the four butterfly species. It is not surprising that most butterflies preferred red and/or orange, because red photoreceptors are much more common in the Lepidoptera compared with other insect species (Briscoe and Chittka, 2001). For example, *P. rapae* and four species of the genus *Lycaena* have four types of photoreceptors that cover a broad spectrum of wavelengths, from UV to red [data for *I. leuconoe* and *A. hyperbius* or their relatives are not known (Briscoe and Chittka, 2001)]. The innate colour preferences of lepidopterans in the context of foraging are often yellow, blue and sometimes orange-red (Weiss, 2001). A preference for white, as found in *A. hyperbius*, may be a rare case; however, white flowers are not uncommon among flowers for which butterflies forage in the field (Ejima, 1987).

Table 2. ANOVA of the forewing length (A) and lifespan (B)

Source	d.f.	MS	F	P
(A) Forewing length				
Species	3	18915	1935	0.000
Sex	1	27.864	2.851	0.094
Species \times sex	3	22.754	2.328	0.077
Error	132	9.773		
(B) Lifespan				
Species	3	1.014	51.916	0.000
Sex	1	0.000	0.008	0.929
Species \times sex	3	0.000	0.006	0.999
Error	96	0.020		

Species refers to the effect of the four butterfly species (*Idea leuconoe*, *Argyreus hyperbius*, *Pieris rapae* and *Lycaena phlaeas*). Sex refers to male and female effects. Significant differences are in bold.

This study has revealed for the first time that learning ability does differ, specifically in flower colour learning rate, during flower foraging among butterfly species (Table 1, Fig. 3). Furthermore, our study corroborated the hypothesis that larger and/or longer-lived butterfly species exhibit increased learning ability (Table 3).

This hypothesis purports that larger butterflies should be better learners because they generally need more nectar than smaller individuals and therefore must forage more efficiently. Another plausible explanation for why larger species should be better learners is that larger species have larger brains, which are associated with better learning abilities. In bumblebees, larger workers have larger brains (Mares et al., 2005) and are also faster learners than their smaller sisters in a colour-learning paradigm (Worden et al., 2005). Although the above study of bumblebees demonstrates an intraspecific correlation between brain size and learning ability, an interspecific correlation among insect species is as yet unknown. It is tempting to assume that learning requires a central nervous system of some minimum size and sophistication (Dukas, 2008). However, despite their relatively small brains, both fruit flies (20,000 neurons) and roundworms (302 neurons) demonstrate associative learning abilities (Dukas, 2008).

Longer-lived butterflies should also be better learners if there are costs associated with the development of cellular mechanisms that enable learning and memory. This is because over their long lifespan they will have more opportunities to switch the flower species on which they rely as floral resources change with the seasons, i.e. longer-lived butterflies should repeatedly employ their learning abilities, enabling them to gain more benefits than costs of learning. Such costs of learning have been reported in fruit flies, whereby flies from lines selected for improved learning ability had reduced larval competitive ability (Mery and Kawecki, 2003), lower egg-laying rates (Mery and Kawecki, 2004) and decreased lifespan (Burger et al., 2008) compared with flies from unselected lines.

According to Lewis and Lipani (Lewis and Lipani, 1990), all butterflies can be roughly divided into two groups: short-lived and small butterflies, and longer-lived and larger butterflies. They predicted that the former butterflies would choose flowers of a given species, following a simple threshold rule whereas the latter butterflies would choose and revisit the most rewarding flowers. They also argued that larger, longer-lived butterflies would have larger brains relative to body size than would smaller, short-lived butterflies, and they could possibly learn locations and landmarks, unlike their smaller counterparts. The authors did not mention that larger, longer-lived butterflies should have superior learning abilities compared with small, short-lived butterflies, although their argument suggests this. Our study was consistent with their expectations and implications, i.e. the larger and longer-lived species *I. leuconoe* and

A. hyperbius exhibited good learning abilities whereas the smaller and shorter-lived species *P. rapae* and *L. phlaeas* had poorer learning abilities by comparison.

This study also demonstrated a difference in learning abilities for flower colour between the sexes, i.e. female butterflies exhibited a higher learning rate for flower colour than males (Table 1, Figs 2 and 3). In other insects, appetitive learning abilities are sometimes different between the sexes (Church et al., 2001; Hartlieb et al., 1999; Kroutov et al., 1999) and sometimes they are not (e.g. Takasu et al., 2007). In bumblebees, Church et al. discovered that female workers learned to discriminate rewarding flower patterns better than males (Church et al., 2001). They suggested that this difference may reflect the differences in their roles within the colony, as the workers might have more of an incentive or disposition to collect food. In the butterfly *Agraulis vanillae*, Kroutov et al. showed that female butterflies were more conditioned than conspecific males to chemical stimuli associated with food (Kroutov et al., 1999). They suggested that the complexity of female behaviour, such as locating oviposition sites and determining host-plant suitability, may enhance learning in females. However, we propose another hypothesis to explain the better learning in female butterflies. We hypothesise that female butterflies require more nectar, compared with their male counterparts, to produce and oviposit eggs. Therefore, they must be able to forage more efficiently, which enhances their learning abilities. For example, female *P. rapae* consumed approximately twice the amount of nectar per day than conspecific males when they were allowed to freely mate and oviposit in the outdoor cage [35.74±6.88 mg (mean ± s.d.), *N*=8, for females; 18.29±3.49 mg, *N*=8, for males (I.K., unpublished)]. Field observations revealed that females of *P. rapae* and *P. napi* exhibited flower foraging behaviour more than twice as frequently as conspecific males [34.4 and 13.8% of various behaviours in *P. rapae* females and males, respectively; 25.8 and 11.0% of behaviours in *P. napi* females and males, respectively; calculated from table 5 in Yamamoto (Yamamoto, 1983)]. These data support our hypothesis; however, further investigations are necessary with regard to sexual differences in nectar consumption and time allocated to nectar collection in other butterfly species.

Another factor that may affect butterflies' foraging behaviour is a preference for nectar amino acids. Female butterflies of some species prefer nectar sources containing amino acids (e.g. Alm et al., 1990; Erhardt and Rusterholz, 1998). One reason for this preference is that amino acids in nectar positively affect fecundity (Mevi-Schütz and Erhardt, 2005). The preference for amino acids can be affected by several factors, such as larval food condition (Mevi-Schütz and Erhardt, 2003) and lifetime mating frequency (Mevi-Schütz and Erhardt, 2004). Such differences in preferences for amino acids may in turn affect learning performance of butterflies. For example, individuals that prefer nectar amino acids may exhibit a higher learning performance toward flowers providing nectar with amino acids than toward those that do not. The relationship between learning ability and preferences for nectar amino acids warrants further investigation.

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Table 3. ANCOVA of the learning rate, including sex as a factor, and forewing length (A) or lifespan (B) as a covariate

Source	d.f.	MS	F	P
(A) Forewing length	1	0.018	15.278	0.011
Sex	1	0.002	1.710	0.248
Error	5	0.001		
(B) Lifespan	1	0.019	23.349	0.005
Sex	1	0.002	2.818	0.154
Error	5	0.001		

Sex refers to male and female effects. To simplify the model, we excluded flower colour as a factor because it did not affect the learning rate (Table 1). Significant differences are in bold.

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