Learning signals within sensory environments: Does host cue learning in butterflies depend on background?

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Abstract—Insects must detect and interpret stimuli embedded in a sensory environment of competing stimuli. While sensory environments vary in time and space, individuals may be able to learn local background characteristics, facilitating perceptual learning. This study on host search in butterflies examines the following questions in an ecologically relevant context: i) does cue learning depend on the sensory environment in which learning occurs; and ii) are background characteristics learned, such that performance on novel tasks in the same sensory environment is facilitated? Females of *Battus philenor* (Papilionidae: Lepidoptera) were trained to different coloured and shaped oviposition targets, against different background colours. Individuals trained to colours on a brown background but tested on a green background performed significantly worse than control individuals which were trained to the same colours but on a green background. Females pre-trained to discriminate green targets from red targets on a green background colour performed significantly better in a novel task (shape learning) involving green shapes on a green background than did individuals trained to discriminate the same colours on a brown background. These two results were unique to particular cue-background combinations, in particular cryptic conditions. Taken together, our results suggest that cue learning depends on an insect's sensory environment, and that learning characteristics of local backgrounds may confer benefits to habitat-faithful individuals.

Keywords: butterflies; learning; background; *Battus philenor*; context-dependency; crypticity; filters; sensory noise; signal detection.

INTRODUCTION

Organisms do not detect signals and cues in a sensory vacuum; such stimuli occur against a background of competing stimuli. The background, or sensory environment, includes all stimuli within an environment, including irrelevant stimuli

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termed 'noise', which are similar to, and thereby often obscure, stimuli of interest. Sensory environments are moreover inherently variable over time and space. For example, different habitats are characterised by visual environments that vary in terms of the spectral composition of ambient light and the reflectance properties of vegetation and substrates (Endler, 1993; Marchetti, 1993; Endler and Théry, 1996; Leal and Fleishman, 2004). Ambient sound levels also vary significantly between habitat types, some habitats being consistently noisy over certain frequency ranges (e.g., Morton, 1975; Ryan and Brenowitz, 1985; Slabbekoorn and Smith, 2002). Acoustic noise due to sound reverberation varies between seasons in deciduous forests (Naguib, 2003) and between habitats (Richards and Wiley, 1980). Phytophagous insects must discriminate host plants against olfactory cues from non-host plants: olfactory background characteristics depend on factors such as non-host plant species present, vegetation structure, weather conditions, and, in an agricultural context, the diversity and spacing of crops (reviewed by Visser, 1986).

Species often adapt to cope with characteristics specific to particular sensory environments. For example, signals used in communication evolve to travel efficiently through particular habitats (e.g., Ryan and Brenowitz, 1985; Marchetti, 1993; Endler and Théry, 1996; Slabbekoorn and Smith, 2002; Cunningham et al., 2002; reviewed by Wiley, 1994). Sensory systems evolve not only to perceive relevant information (Wehner, 1987) but also to filter out irrelevant background information (reviewed by Endler, 1992).

Additionally, individuals may dynamically adjust signals to spatiotemporal variation in sensory environments. Such individual level responses have been best studied from the perspective of the signaller in communication systems. When signals are obscured by the background, signals tend to increase in intensity or in number of repetitive elements (Cynx et al., 1998; Lengagne et al., 1999; Brumm and Todt, 2002; Brumm, 2004). Alternatively, signallers may opt to signal later, when background conditions change (e.g., delays in communication due to weather conditions (Lengagne and Slater, 2002), or time of day (van Staaden and Römer, 1997)).

Individual-level plasticity in response to sensory environments has been much less studied from the standpoint of a signal receiver. However, a receiver may also respond to variation in the sensory environment, whether with respect to signals from conspecifics, or cues from predators or prey. Receivers could cope with variability in sensory environments in several, non-mutually exclusive ways. For instance, signal detection theory predicts that receivers may adjust their threshold of response to a stimulus of interest so as to simultaneously maximise responses to the relevant stimulus and minimise responses to similar, but irrelevant, stimuli (so-called 'false alarms'). A signal detection framework (cf. Wiley, 1994) has recently been applied with success to analysis of learning of floral cues by foraging bumblebees (Lynn, Cnaani and Papaj, 2005).

Alternatively, individuals experiencing a particular sensory environment may learn, through a process of perceptual learning, to ignore features of the environment and thus improve subsequent performance (Watanabe et al., 2001). Perceptual

learning, long studied in psychology (reviewed by Sathian, 1998; Goldstone, 1998), has been documented in many non-human animals; for instance, in the formation of search images during search for cryptic prey (Pietrewicz and Kamil, 1979; Plaisted and Mackintosh, 1995; Langley, 1996; Goulson, 2000; Bond and Kamil, 2002) and in learning of olfactory cues during search for host plants (reviewed by Visser, 1986; reviewed by Papaj and Prokopy, 1989). Theory and experimental evidence from cognitive science and psychology predict that perceptual learning involves not only learning templates for the cues, but also learning to ignore aspects of the sensory environment (Vaina et al., 1995; Dosher and Lu, 1998, 2000; Sigala and Logothetis, 2002; Gold et al., 2004; Yang and Maunsell, 2004). However, studies of such 'background learning' in an ecological context are rare.

This study addresses the hypothesis that, in an ecological context, insects learn not only cues associated with rewards but also the means by which to extract those cues from the sensory environment. We predicted that: i) the efficacy of response to a given cue in a given sensory environment will depend on the sensory environment in which that cue was learned; and ii) characteristics of particular backgrounds are learned and transferred to learning of novel tasks in that sensory environment. Our predictions were tested in the context of visual cue learning by host-searching butterflies, where the 'sensory environment' is the visual background surrounding the host cues.

METHODS

Study system

The pipevine swallowtail butterfly *Battus philenor* L. is a papilionid species common in North America that specialises on *Aristolochia* species over its entire range. In southern Arizona the butterfly uses a single host species, *Aristolochia watsoni* Wooton & Standley. The *Battus philenor-Aristolochia watsoni* system is a particularly appropriate study system for the questions asked here, being characterised by considerable variation both in the host resource and the vegetative background against which the host occurs. In southern Arizona *B. philenor* is active between March and September, including a dry, pre-monsoon period (March-June) and a rainy, monsoon season (July-September). Throughout the year, the small, highly recumbent host plants vary markedly in leaf colour, ranging from a dark red to a bright green; some plants are consistently one colour while others switch colour, and still others consist of mixtures of red and green foliage. All forms can occur at a given site, with green forms always rare but becoming more common after the monsoon. The vegetative background in the mesquite-grassland habitat consists largely of grasses and herbaceous plants, and changes visually from brown and yellow before the monsoon, to green after the monsoon. Additionally, host plants in washes commonly appear against brown backgrounds independent of season, adding a component of spatial heterogeneity in background. Host-searching females

frequently land on non-host plants in the vicinity of host plants, suggesting that the vegetative background against which hosts are found might influence host finding.

Battus philenor readily learns oviposition cues, including colour and some aspects of shape (Papaj, 1986; Allard and Papaj, 1996; Weiss and Papaj, 2003), and performs well under controlled laboratory conditions. Females have been demonstrated to use colour as a host-finding cue in the field and to learn red or green readily under laboratory conditions (Weiss and Papaj, 2003; D. Papaj, unpubl.).

Study subjects were obtained from a laboratory colony. Larvae were reared on fresh *Aristolochia fimbriata* leaves replaced daily (13:11 L:D photoperiod, 23°C). Adults were transferred to a large flight cage (2 × 2 × 2 m) and hand-fed one to three times daily on a 15% honey solution. Four 500 W halogen lights provided heat and light for courtship for several hours per day. Mated females were individually numbered on their wings with a gold paint pen. Except during oviposition training, mated females were kept naïve in terms of host experience by placement in holding cages (0.25 × 0.25 × 0.25 m) within the flight cage. Training sessions lasted 1.5-4 h, depending on how long it took females to reach training criteria. Testing and shape training sessions occurred between 2 and 48 h following training and lasted 2-4 h. Females used in the experiment were fed before and after training or testing sessions. In general, females were completely host-naïve prior to training; however, some females were provided limited access to a host plant, a live *Aristolochia watsoni*, so as to relieve egg load. In ANOVAs including treatment group, prior experience with a host plant had no effect on training performance ($F_{1,28} = 0.86$, P = 0.36) or test performance ($F_{1,28} = 0.16$, P = 0.69).

Oviposition learning

Butterflies were tested on an array of 16 host plant models, hereafter referred to as 'targets', arranged in a Cartesian grid, with targets spaced 20 cm apart. Oviposition targets were constructed from paper and consisted of six rectangular 'leaves' projecting radially out from an inverted plastic pipette tip. Targets were 6 cm diam. Each oviposition target was placed in the centre of a 20 cm^2 paper square of a certain background colour (green or brown). A square of this size ensured that the background was visible to a butterfly from behind the target at an angle of approach as low as 25°. Oviposition targets were red, green, or blue. Target colour was generated by printing on white inkjet paper (waterproof, 'National Geographic Adventure paper', Teslin$^®$) from an Epson Stylus C80 inkjet printer using Durabrite brand inks.

Red and green targets were spectrally matched to natural variation in host plant colour using an Ocean Optics S2000 spectrophotometer. Blue was an arbitrary colour that was similar in peak intensity to the green target. In Experiment 1, probably the most cryptic treatment group consisted of green targets against a green background, as the hue (wavelength of peak intensity) of both the target and the background is closely matched. In Experiment 2, the green background colour was darkened so that the green target was less cryptic against the green background
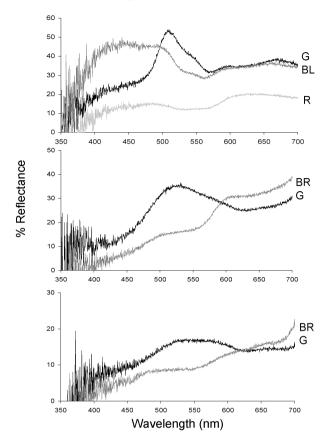
Figure 1. Spectral reflectance of targets and backgrounds. Each graph shows the reflectance (y axis) for each wavelength (x-axis, nm). The top graph includes each target used (G = green, R = red, BL = blue), the middle and bottom graph show the backgrounds used in Experiments 1 and 2, respectively (G = green, BR = brown).

(fig. 1), making the treatment groups of comparable crypticity and to encourage shape learning, a more difficult task than colour learning.

During training, each target had a central cotton wick, wet either with water plus 150 μl of *Aristolochia fimbriata* extract (in the training mode, S+) or with water tinted to the same orange colour as the extract (in the neutral mode, S_0). The extract was prepared by blending 385 g fresh *A. fimbriata* leaves in 675 ml boiling ethanol. The blended solution was filtered under vacuum and ethanol removed under vacuum at 40°C until the extract was concentrated to 400 ml. This resulted in a concentration of 1 g of *Aristolochia* foliage per ml solvent, or 1 g leaf equivalent (= 1 gle). The resulting, mostly aqueous extract was centrifuged to remove chlorophyll as a particulate. The decanted solution was stored in sealed glass containers at −4°C. Wicks were changed daily.

Rewarding and neutral targets were stored separately to prevent cross-contamination. In both experiments, position data were recorded to test for spatial location biases (none were found).

Learning assays

Training was initiated either by placing females directly on an S+ target or waiting for a female to begin host searching spontaneously. Direct placement generally elicited a highly stereotyped, oviposition search behaviour, characterised by a slow, fluttering flight, frequent turns, and periodic landings on targets. While number of placements was not strictly controlled, varying with individual lifetime and other factors beyond our control, the number of placements per individual did not differ among the four training groups ($F_{3,42} = 1.63$, P = 0.19).

Once a female was in oviposition search mode, each landing on a target by that female was recorded with reference to target number and colour. If the target was probed (for nectar) the landing was not counted, and the individual was immediately fed. If the individual landed on the target and basked, the landing was also not counted. Landings on the background in proximity to targets were recorded. Successive landings on the same target were recorded as separate landings only if, between landings, the individual left the square with the target of interest and paused in flight over other targets.

During test phases, individuals were induced to search for hosts by placing them on a wick (separate from any targets) wetted with *Aristolochia* extract and/or placing them directly on a neutral target. During all observations, a wick wetted with water and 1 ml crude *Aristolochia* extract was placed nearby such that volatiles were likely to stimulate females to search.

Data analysis

To obtain accurate estimates of initial errors and learned responses, we used logistic regression as a statistical model of change in behaviour due to learning. Logistic regression was applied to the landing data for each individual, yielding regressions with P values of 0.10 or less for 20 of 44 individuals trained for at least 20 landings (Exp. 1) and nine of 23 trained for at least 50 landings (Exp. 2). Using the equation for logistic regression (Eq. 1) and the output estimates for the regression parameters (a, b), the probability of correct landings (y) was calculated for each individual both for initial probability of error ($x = 0$), referred to as 'initial error' and final probability of error ($x = $ total landings for that individual), referred to as 'final error' (see fig. 2).

$$y = \frac{1}{1 + e^{-(a+bx)}} \tag{1}$$

'Training performance' was defined as the difference between final error and initial error (fig. 2); 'test performance' was defined as the difference between the test error
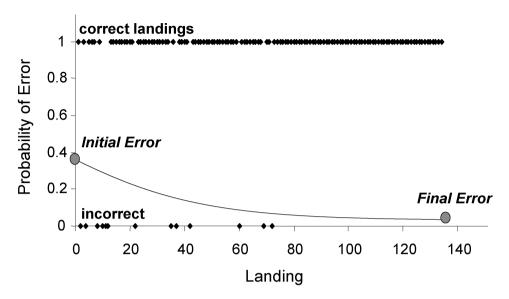
Figure 2. A representative learning curve during training. Points represent individual landings on either the correct, rewarding target ($Y = 1$) or the incorrect, non-rewarding target ($Y = 0$). A logistic regression is fit to the data to represent the decrease in probability of error over time. Two parameters from this regression, the initial error (error at $X = 0$ landings) and the final error (error at $X =$ final landing) are used in the analysis. This individual was trained to a green target against a green background.

(percentage of incorrect landings throughout the test) and the final error during testing (as calculated from logistic regression). All analyses involving proportions (e.g., initial errors) were arcsine square root transformed prior to analyses.

EXPERIMENT 1: ARE CUES LEARNED INDEPENDENTLY OF THE BACKGROUND?

Experimental design

To determine if cues are learned independently of background, individuals were trained either to a red or a green target type against either a green or brown background, yielding four training groups: i) Green/Green (S+/Background); ii) Green/Brown; iii) Red/Green; or iv) Red/Brown (see fig. 4). Individuals from each training group were tested against either a green or brown background, yielding a total of eight groups of butterflies. At least five butterflies were used in each of the eight groups. The four groups consisting of individuals trained and tested against the same background were considered control groups. The other four groups consisting of individuals trained on one background but tested against the other background were considered treatment groups. During the testing session, test targets had wicks wet with orange coloured water.

Learning criteria

Individuals were trained for at least 20 landings on either target (mean = 69.17 (SD = 47.33), N = 45, range = 20-234). Total landings did not differ among the four training groups ($F_{3,41}$ = 0.96, P = 0.42) or eight test groups ($F_{7,26}$ = 0.70, P = 0.67). Training lasted for at least 20 landings, until performance improved by at least one landing between the first and final ten landings. Following training, logistic regression was used to confirm that trained individuals actually improved in performance overall. If the 'training performance', the difference between the final error and the initial error, was at least 0.05, the individual was included in the analysis. This protocol eliminated individuals that did not improve their performance during the training session, and reduced sample sizes slightly between groups: 1C, 1T, 2C, 2T, 3C, 3T, 4C, 4T; N = 5, 5, 4, 4, 4, 3, 4, 5. Using this criterion, individuals included in the analysis reduced their error rate by an average of 31 percentage points (SD = 19, N = 44, range = 5.1-69.9 percentage points).

RESULTS (1)

Initial error and learning during training

Butterflies learned over the course of training to land preferentially on the rewarding target. Data from a typical training session are shown in figure 2 with a logistic regression fit to the landings to describe changes in the probability of error. Among all butterflies trained, the difference between initial and final error was significantly greater than zero, indicating that the rewarding colour was learned (t_{50} = 8.67, P < 0.001). Training performance (= the difference between initial and final error) was related to background colour, with stronger performance against brown backgrounds (fig. 3, background parameter: $F_{1,40}$ = 5.43, P = 0.03); performance was not related to the S+ colour or to an interaction between target and background colour.

The butterflies' initial error, standardised with reference to a green target (i.e., 1 = all green landings, 0 = all red landings), was affected by the background (background parameter: $F_{1,26}$ = 5.61, P = 0.02; S+ and interaction NS); the chance of choosing green being significantly higher against a brown than a green background (fig. 3). There was no difference among the eight test groups ($F_{7,26}$ = 1.49, P = 0.22) or between control and treatment groups in initial error for green. There was no difference between control and treatment groups in training performance except for a marginal difference in the red on brown group (P = 0.08) where the treatment group had higher training performance than the control group (fig. 3); however, this difference was in a direction opposite that which might bias results.
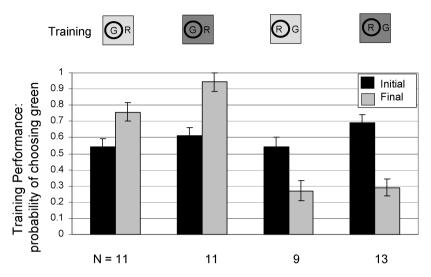
Figure 3. Summary of training performance. The Y-axis represents the initial (black bars) and final (grey bars) errors with respect to the green target. While statistical tests were performed on transformed values, untransformed data are shown; error bars represent standard error. All individuals start at comparable initial errors (slightly biased towards green), and diverge as they learn to choose only the green or only the red targets. Each column represents a different training group of butterflies as labelled above: light and dark grey backgrounds represent green and brown backgrounds, respectively; 'G' and 'R' represent green and red targets, respectively; dark circles signify the rewarding target (with host plant extract).

Effects of background on memory

The difference between an individual's test error (= total percentage of incorrect landings during the test) and its final error during training was significantly affected by treatment in addition to target colour, training background, and test background (fig. 4, overall model: $F_{4,29} = 11.18$, $P < 0.0001$). As evidenced by a significant training background × test background interaction effect ($F_1 = 9.33$, $P = 0.005$), performance on a given test background depended on treatment group. Individual t tests showed that individuals that switched from a brown training background to a green test background had significantly lower test performance relative to controls, both for green target training (fig. 4; $P = 0.009$) and for red target training (fig. 4; $P = 0.02$).

Performance for individuals trained to green targets deteriorated less between training and test sessions than performance for individuals trained to red targets (S+ parameter effect: $F_1 = 13.9$, $P = 0.008$). Performance for individuals trained on a green background deteriorated less than performance for individuals trained on a brown background ($F_1 = 9.61$, $P = 0.004$). Performance for individuals tested on a green background generally exceeded that of individuals tested on a brown background ($F_1 = 6.20$, $P = 0.02$). Remaining interaction terms in our fully-factorial ANOVA were not statistically significant ($F < 0.40$, $P > 0.50$).
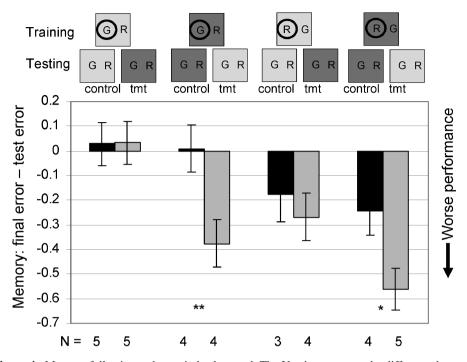
Figure 4. Memory following a change in background. The Y-axis represents the difference between the error at the end of the training session (final error) minus the total error during the test (with respect to the target colour the individual was trained to). The X-axis signifies the training and treatment regime for a butterfly, according to symbols outlined for figure 3; control groups are trained and tested on the same background while "tmt" or treatment groups are trained and tested on different backgrounds. Thus a zero value represents complete retention of learned information between training and testing, and negative values represent 'forgetting'. Treatment groups (those that switched backgrounds) performed worse than control groups only when training was on a brown background and testing on a green background. Error bars represent standard error.

EXPERIMENT 2: ARE CHARACTERISTICS OF THE BACKGROUND LEARNED AND APPLIED TO NOVEL TASKS?

Experimental design

In this experiment, we sought to determine if a female learned characteristics of the background during a pre-training colour discrimination task and transferred this learning to a novel, shape discrimination task. Female butterflies were first trained to discriminate rewarding oviposition target from an unrewarding red target. For one group of females, the rewarding target was green; for another group, the rewarding target was blue. As in Experiment 1, the targets consisted of a six-pronged, radially symmetrical shape, 6 cm in diam. Extracts were applied to rewarding targets as described in Experiment 1. Half of each colour training group was trained against a green (control) or brown (treatment) background colour (see fig. 5). Females in each target colour × background colour combination were then given training on a second oviposition task, involving discrimination between two novel green shapes
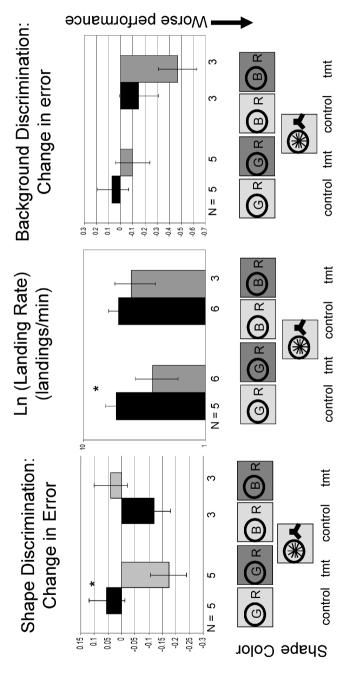
Figure 5. Performance on a novel discrimination task (shape) following experience with particular backgrounds during colour training. The Y-axis represents a particular measure of performance during shape training. The X-axis is as in figure 3 except 'B' represents blue targets, and shapes in the lower box represent the two targets used in the shape training. The left-hand graph shows changes in the correct choice of the rewarding shape over the shape training session (i.e., difference between initial and final errors, see fig. 2), thus more positive values represent improvements in discrimination during shape training. The middle graph shows landing rates on targets during shape training (note log scale), thus more positive values represent higher performance. For these first two measures of performance, the group with previous experience on the shape-training background (control group) performed better than the group without such experience, only when colour training occurred on green targets. The right-hand graph shows changes in ability to ignore the background between colour training and shape training; while individuals learned to ignore the background during colour training (see text), this ability was not retained in shape training as the difference between initial background landing during colour training and shape training was less than or equal to zero (signifying no change, or an increase in background landings between training sessions, respectively).

(a rewarding 12-pronged versus an unrewarding three-pronged target of the same overall area) against a green background.

Several measures of performance during the shape discrimination task were made. First, we measured the total change in discrimination between the two shapes over at least 20 landings (mean no. of landings = 68.3 (SD = 33.55)). Similar to the above analyses of learning, logistic regressions were fitted to the shape training data, where landing on a rewarding target was considered a success (Y = 1) and landing on an unrewarding target was considered an error (Y = 0). Change in shape discrimination was estimated by subtracting the final error in the shape session from the initial error during that session (fig. 2).

The second measure of performance was target landing rate (targets per min) during a searching session. The time, to the nearest second (s) for each landing was recorded during shape sessions to facilitate this calculation. A searching session was started when a female entered oviposition mode (see description above) and ended when the female left the array or was inactive for at least two min. Target landing rate was calculated as the total number of landings, on either rewarding or non-rewarding targets, divided by the total time of the session, averaged over all searching sessions for each individual with at least two sessions. Our measure of target landing rate was skewed towards low values; a natural log transformation was used to normalise the distribution.

The third measure of performance was discrimination against background. Landings on the background as well as landings on targets were recorded during both the colour and the shape training phases. Logistic regressions were computed to determine if individual butterflies learned to discriminate targets against the background: target landings were counted as successes (Y = 1), and background landings were counted as errors (Y = 0). We used parameters from logistic regression to test if background discrimination improved during colour training and if this discrimination was retained during shape training. If butterflies learn features of the background, they should learn and remember to ignore (i.e. not land on) the background.

We predicted that during shape training, individuals with colour training against a green background would, relative to individuals colour trained against a brown background: i) learn to discriminate shape faster; ii) have higher shape-training target landing rates; and iii) learn to ignore the green background during colour training and retain this response during shape training. If females learn background characteristics dependent on characteristics of the cue, these predictions should hold only when background and cue colours remain the same between discrimination tasks (i.e. the green/green colour task and green/green shape task).

Learning criteria

The protocol for quantifying and analysing learning was identical to that of Experiment 1. Individuals with at least 50 landings in colour training (and a difference in at least one landing between the first and last ten landings) were

advanced to shape training. Individuals were included in the analysis of shape learning rate if they had at least 20 landings during shape training. There was no difference between treatment groups in total number of shape-training landings ($F_{3,12} = 0.33$, P = 0.80). Individuals were included in the analysis of target landing rate if there were at least two searching sessions during shape training for which landing rate could be calculated.

RESULTS (2)

Learning during colour training

Overall, during colour training, females learned to choose the rewarding target over the unrewarding target as their final error rate was significantly lower then their initial error rate ($t_{22} = 3.83$, P = 0.0009). There was no significant difference between the four colour training groups in training performance (initial error-final error, $F_{3,19} = 1.31$, P = 0.31) or in initial error ($F_{3,19} = 2.25$, P = 0.12) although there was a trend for initial error to be highest in the green against green treatment and lowest in the blue against brown treatment.

Learning of shape

We measured, over the course of shape training, changes in frequency of landing on the rewarding novel shape (12-prong versus three-prong target). In contrast to learning target colour, individuals did not learn overall to discriminate between the two shapes because the difference between their initial and final errors was not significantly greater than zero (mean (SE) = 0.053 (0.04), $t_{15} = -1.3$, P = 0.20). Rather, changes in response to shape depended on treatment. Individuals colour trained to green targets against a green background improved significantly more in shape discrimination than did individuals colour trained to green targets on a brown background (fig. 5; $F_{1,8} = 5.93$, P = 0.041). In contrast, individuals colour trained to blue targets against a green background improved less in shape discrimination than individuals colour trained to blue targets on a brown background; however, this difference was not statistically significant (fig. 5; $F_{1,4} = 3.84$, P = 0.12). Taking these results together, colour training on a target colour/background colour appeared to facilitate shape learning on the same target colour/background combination.

Target landing rate during shape training

The interaction between target colour and treatment group on shape discrimination was paralleled by an interaction in terms of landing rate on each target during the shape training session (fig. 5). Individuals colour trained to green targets against a green background enjoyed significantly higher target landing rates in the shape training phase than individuals trained to green targets against a brown background (fig. 5, P = 0.03). In contrast, individuals colour trained to blue against

a green background had target landing rates in the shape training phase that were virtually identical to individuals colour trained to blue against a brown background (fig. 5, P = 0.76). While treatment group alone (colour training background) was marginally significant in explaining variation in target landing rates during the shape training ($F_{1,18}$ = 4.19, P = 0.055), the overall statistical model for landing rate did not identify training target colour, treatment, or the interaction, as significant effects, possibly due to low power (background colour: $F_{1,16}$ = 3.04, P = 0.10, target colour: $F_{1,16}$ = 0.08, P = 0.78, target × background: $F_{1,16}$ = 1.64, P = 0.22). In conclusion, while sample size is limited, results suggest that experience with a green background increases target landing rate in a novel task against a green background when butterflies are colour trained on green targets, but not blue ones.

Learning to discriminate against background

We tested whether females learned to avoid landing on the background colour during colour training, and whether this discrimination was remembered during shape training. Overall, individuals significantly improved their ability to avoid landing on the background during colour training (t_{24} = 3.31, P = 0.003), decreasing the estimated probability of landing on the background almost three-fold on average, from 0.30 (SE = 0.047) to 0.11 (0.025). However, individuals did not retain this ability to avoid the background between colour training and shape training. For each individual, we used logistic regression to estimate the initial probability of landing on background during colour training; we used a separate logistic regression to estimate the initial probability of landing on background during shape training. We found that the initial background landing error during shape training was equal to or higher than the initial error during colour training (fig. 5).

The difference in frequency of background landing errors between colour training and shape training sessions depended on target colour. In a full statistical model, treatment group (experience with shape-training background colour) had no effect on changes in background landing frequency between colour and shape training, while target colour was marginally significant: individuals colour trained to blue targets had higher error rates during shape training than colour training (effects on difference between initial error in colour training and error during shape training: target colour: $F_{1,12}$ = 4.44, P = 0.056, colour training background: $F_{1,12}$ = 2.94, P = 0.11, interaction NS). In summary, our analysis of responses to background suggests that: i) females learn to discriminate against the background; ii) this discrimination ability is not remembered in a new context; and iii) increases in background landing mistakes during a novel task are especially high for individuals lacking experience with the background colour of the novel task (either green targets or a green background).

DISCUSSION

This research explored the relevance of variation in sensory environments to foraging behaviour: i) are cues learned independent of the sensory environment; and ii) are features of the sensory environment learned and used to advantage in learning novel tasks? We address each question in turn in the next two sections.

Are cues learned independently of the sensory environment?

For host-searching butterflies, the answer appears to be 'no'. In this study, cue learning depended on the sensory environment in which cues are experienced. When female butterflies were trained to either red or green targets against a brown background, their performance on the colour task was worse when tested subsequently on a green background, relative to control individuals tested subsequently on the same brown background (fig. 4). In contrast, for females trained against a green background, there was no effect of switching to a brown test background.

These results suggest that butterflies learn characteristics of a green background. Changes in the relative conspicuousness of the rewarding targets (fig. 1) cannot explain these results because the pattern held for both red and green rewarding targets (fig. 4). That a change in background signals a new context (Lotto and Chittka, 2005), and the irrelevance of previously-learned associations, cannot explain the results either, because changes to green but not to brown backgrounds resulted in less (or no) retention of learned associations (fig. 4).

Several non-mutually exclusive mechanisms can explain why learned cues can be extrapolated from green to brown backgrounds, but not from brown to green ones. First, whether or not females attend to the background during learning may depend on the overall conspicuousness of *both* targets. In training against a green background, the green target (sometimes S+, sometimes S_0) appears somewhat cryptic as the hue (wavelength of peak reflectance) of the target closely matches that of the background (fig. 1). Thus, under cryptic conditions, females may learn background characteristics permitting them to discriminate the green target from the green background, whether the green target is rewarding or not. In training against brown, by contrast, both targets appear to be more conspicuous (fig. 1). For this reason, background characteristics may not be learned, or learned as well, for cues against brown backgrounds.

Alternatively, the result may relate less to crypticity of targets and more to the fact that green is intrinsically highly stimulating to females engaged in host search (e.g., initial error rate is biased towards green, fig. 3). The added stimulation in the green background may somehow disrupt discrimination between targets when females have been trained on a non-stimulating brown background. In this case, experience with the green background permits females to exclude the background as a candidate for host tissue. A brown background, on the other hand, does not represent a potential host, and consequently does not disrupt discrimination between targets. Distinguishing between these two mechanisms would require manipulating

crypticity independently of intrinsic stimulation (for example, adding a training treatment using red targets against red background).

Are characteristics of the background learned and applied to novel tasks?

The answer appears to be a qualified 'yes'. Experiment 2 provided evidence that some characteristics of the background are learned and applied to novel tasks, but that such learning is selective. Training to a green colour against a green background facilitated improved performance in a novel shape discrimination task of the same target colour-background colour combination (fig. 5), compared to individuals with training to a green colour against a brown background. In contrast, individuals trained to a blue colour showed no clear effect of training background on learning of the shape discrimination task (fig. 5). We tested for a possible mechanism involving learning and remembering to avoid landing on the background. While individuals learned to refrain from landing on the background during training to colour, they were apparently unable to retain this discrimination when transferred to a shape training task (fig. 5). Hence, the component of background learning that may be transferred to learning of a novel task involves something different than learning to refrain from landing on the green background.

Several non-mutually exclusive mechanisms can explain why background learning appears to be adopted under training to green against green, but not under training to blue against green. First, background learning may occur only under cryptic conditions. However, we note that, in Experiment 2, green against green was less cryptic than in Experiment 1 because the background was darker (see fig. 1). Second, background learning may occur only when the background colour is innately attractive (green) and must therefore be actively ignored during host search. As above, these hypotheses could be distinguished by manipulating crypticity independently of intrinsic stimulation (for instance, adding a red against red treatment condition in the first training phase). Finally, because perceptual learning is often highly specific (reviewed by Sathian, 1998), background learning may only be extrapolated to novel tasks when characteristics of both the target and the background are similar between tasks (i.e. green targets and green backgrounds, fig. 5).

Perceptual learning and sensory environments

Perceptual learning involves learning to detect objects of interest, be they food items in foraging or conspecifics in communication. The present study suggests that perceptual learning is dependent on sensory environment (fig. 4) and that learning characteristics of the background occurs simultaneously with the learning of cues (fig. 5). Our results are broadly applicable to other systems in which perceptual learning has been studied. For example, search image formation, as in birds learning to detect cryptic prey (Pietrewicz and Kamil, 1979), is considered to be a kind of perceptual learning. Traditionally, discussion of search image formation has emphasised the learning of features of the prey per se. Our results suggest that search

image formation may involve learning of prey cues *and* learning of background features, as suggested by some theory and empirical evidence in neuroscience and psychology (Dosher and Lu, 1998, 2000; Sigala and Logothetis, 2002; Gold et al., 2004; Yang and Maunsell, 2004).

If search image formation involves learning both of prey and of background, our perspectives on prey strategies to defeat search image formation may need to be broadened. For example, it has been proposed that cryptic prey benefit by being variable in phenotype (Bond and Kamil, 2002). Our present results, if generalisable, suggest that prey might also benefit by occurring against varying backgrounds.

This research also has relevance for a phenomenon in the search image literature known as background cuing. When cryptic signals are associated with certain background environments, animals appear to use the background to prime a search image for the associated signal; this form of learning is called background cuing (Kono et al., 1998). The results of this study suggest that there may be inherent mechanisms in perceptual learning to account for background cuing: the sensory environment appears to be learned in association with cues, especially when cues are cryptic (figs. 4, 5).

Our perspective on the value of learning from the standpoint of the predator, or the herbivore, may need to be broadened as well. For instance, it is well known that the learning of one task can facilitate learning of novel tasks (Shettleworth, 1998). Our results suggest that one means by which such facilitation can occur is through learning of background characteristics which are transferred to other tasks. Such extrapolation may be relevant to insect learning in nature. For instance, a bee or butterfly's learning to ignore the background in foraging for one flower type (Goulson, 2000) may facilitate learning subsequently of another flower type in the same or similar sensory environment.

Future directions

In the *Battus-Aristolochia* system in southern Arizona, we are only beginning to understand the nature of sensory variation in the habitat in relation to variation in visual host plant cues. In nature (and in contrast to our experimental conditions), both green forms and red forms of the host *A. watsoni* are generally highly cryptic to the human observer, albeit for different reasons. Green forms are cryptic against green foliage, a crypticity which increases markedly after summer monsoon rains; in contrast, red forms are so dark as to 'hide' among the shadows of vegetation, soil and rock. What is known about papilionid vision (reviewed by Arikawa, 2003) suggests that *Battus* females must also cope with some degree of visual noise for each colour form. If so, it may benefit females to learn not only the various colours of host tissue, but also elements of the background against which each colour form occurs. Certainly, anyone who watches females engaged in host search in the field gains an immediate sense that females would benefit by learning features of the background. This is because females identify host plant in part by tasting the leaf surface with chemoreceptors on their foretarsi. In the field, host-searching females

alight briefly but frequently on objects that are not *Aristolochia* plants, including mainly non-host vegetation but also dead twigs, dirt and stones. In fact, such 'mistaken' landings, which must consume time and may increase vulnerability to ground-borne predators, are far more numerous than landings on actual hosts (by as much as two orders of magnitude; D. Papaj, unpubl.).

Apart from understanding the relevance of this study for this particular insect-host interaction, the study needs to be repeated in other systems and with larger sample sizes. Testing multiple sets of cryptic targets and background conditions may clarify the mechanisms underlying background learning. To what extent is the sensory environment dealt with through associative learning versus alternative processes such as sensory adaptation and habituation? Sensory adaptation and habituation, which involve reduced responses to recurrent stimuli at the level of sensory receptors and neural circuits, respectively (Torre et al., 1995; Dalton, 2000), are considered to be strategies for coping with noise (Torre et al., 1995; Pierce et al., 1995), sometimes exerting long-lasting effects (Dalton and Wysocki, 1996; Fischer et al., 2000; Bee and Gerhardt, 2001; Rose and Rankin, 2001; Simonds and Plowright, 2004). It is likely that learning backgrounds reflects a combination of sensory adaptation, habituation, and associative learning of features of unrewarding stimuli, but the relative importance of the processes may vary from one circumstance to the next and from one species to the next.

Apart from the mechanisms of background learning, the results raise many other questions. For instance, if colour learning is not independent of background colour, how does the phenomenon of colour constancy develop in Lepidoptera (Kinoshita and Arikawa, 2000)? Does colour constancy itself involve the integration of learning target colour and learning background colour? What constitutes noise in various sensory modalities and does noise in one modality tend to be more constraining than in other modalities? Are there innate biases in terms of coping with sensory backgrounds? Given that perceptual learning is often highly specific (reviewed by Sathian, 1998), what determines the extent to which background learning can be applied to novel tasks? Ecological and behavioural research should consider the consequences of signals and cues being embedded in a sensory environment.

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