

Flower constancy and learning in foraging preferences of the green-veined white butterfly *Pieris napi*

D. GOULSON and J. S. CORY NERC Institute of Virology and Environmental Microbiology, Oxford

Abstract. 1. Evolutionary pressure should select for efficient foraging strategies, within the constraints of other selective forces. We assess the mechanisms underlying flower choice in the butterfly, *Pieris napi* (L.), which as an adult forages for nectar. Experiments were carried out on a laboratory colony, using artificial flowers of two colours, and replicated on two successive generations.

2. When nectar was freely available from all flowers, equal numbers of butterflies visited each colour, but individual butterflies exhibited flower constancy, showing a strong preference for one colour or the other.

3. Following 3 day conditioning periods in which nectar was available from flowers of one colour only, butterflies responded by developing a preference for this colour, which persisted when both flower colours were refilled. This preference could subsequently be switched to the other flower colour following a further 3 days of conditioning. These are interpreted as adaptive (learned) responses, which would have obvious selective benefits in the field, enabling butterflies to avoid flower species which experience has shown are poor sources of nectar, and to adapt to temporal and spatial changes in nectar availability.

Key words. *Pieris*, butterfly, foraging, flower colour, nectar, learning.

Introduction

Adult Lepidoptera are almost all short-lived and dependent on suitable weather for activity, and within their life span must reproduce and in many cases disperse. These activities are fuelled by energy reserves stored as a larva, which in most species are supplemented in the adults with nectar gathered from flowers. Fecundity of female butterflies may be limited by time available for oviposition, particularly in temperate climates (Jones & Ives, 1979; Wiklund & Persson, 1983; Jones, 1987). Time spent gathering nectar may impinge on time available for searching for oviposition sites, or, in males, for searching for mates. Similarly, if the nutrients in nectar contribute directly to fecundity, the rate of acquisition of nectar will directly affect fitness. We thus predict that butterflies should be efficient foragers.

The phenomenon of flower constancy, familiar to Darwin over a century ago (Darwin, 1876), has provoked considerable interest, for it appears to provide an example of sub-optimal behaviour: flower constant insects tend to

feed exclusively on one species of flower in a foraging bout, and in doing so may ignore flowers which provide a greater reward (Wells & Wells, 1983, 1986; Wells *et al.*, 1992). Honey bees (*Apis mellifera*) have been used widely as a model system to study nectarivore foraging. Individual bees tend to be constant; of up to 500 visits to flowers made in a single foraging trip from the hive, 93–98% of all visits were to the same flower species (Grant, 1950; Free, 1963). The preference of an individual bee depends on past experience. In simple two-choice experiments in which only one choice provides a reward, bees rapidly develop a preference for the correct flower, using scent, colour, shape, or a combination of the three in identification (Wehner, 1967; Koltermann, 1969; Menzel & Erber, 1978). Learned preferences may persist for at least 2 weeks (Menzel, 1967). Switching preferences when rewards are reversed is not as rapid as the initial learning process, eight to ten rewards being required to achieve 90% correct choices using flower colour, compared to three to five rewards for the initial learning process (Koltermann, 1969). However, in two-choice experiments where all flowers provide a reward, but the relative amounts of reward per flower differ, bees do not always switch to the flower which provides the greatest reward (Wells *et al.*, 1992), perhaps

Correspondence: Dr Dave Goulson, NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR.

because of their imperfect knowledge of the distribution of resources. Switches in flower preference were not found in solitary bees which have a short adult life span (Heinrich, 1976).

Studies of insect nectarivore foraging are, with one notable exception, confined almost exclusively to Hymenoptera. However, Lewis (1986, 1989; Lewis & Lipani, 1990) described foraging strategies very similar to honeybees in the butterfly *Pieris rapae*, suggesting that flower constancy may be more widespread than previously suspected. This study examines foraging strategies in the green-veined white butterfly *Pieris napi* (Lepidoptera: Pierinae): we test for flower constancy, and whether individual butterflies are able to adjust their flower choice in response to previous successes. Studying behaviour in the laboratory enabled conditions to be precisely controlled, and allowed for replication of observations on the same individuals over a large portion of their life span, which cannot be achieved in the field. Colour was chosen as the variable flower characteristic in preference to shape, which produces a weak response in bees, or scent, which is more difficult to manipulate.

Materials and Methods

Thirty-two wild females caught in the University Parks, Oxford, were induced to oviposit in captivity, and the resulting first and second generations of offspring used for experiments.

Experiments were carried out in a 80 cm × 48 cm wide × 50 cm high Perspex cage, with ventilation and access provided by a 30 × 30 cm netting panel. A strip of tissue paper was hung from each end of the cage to provide a suitable

surface for the butterflies to rest on. Illumination was provided by four 20W fluorescent light strips for 16 h per day, and the temperature maintained at a constant 21°C. Potted plants of garlic mustard *Alliaria petiolata* (Cavara & Grande) (Cruciferae) were provided for oviposition, but removed during experiments. Nectar was supplied by eight artificial flowers, four blue and four red (Bright blue and Bright red, Hi grade enamel, J. Perkins, London) containing 10% honey solution (henceforth described as nectar). Measurement of the spectral properties of the artificial paints was not carried out (Menzel & Shmida, 1993), for the experiments only required that the insects be able to distinguish the alternative colours. Flowers were constructed from a 3 cm radius disc of painted cardboard, with a plastic Eppendorf tube inserted through a hole in the middle to contain the nectar. These were raised 30 cm above the floor of the cage on wire supports, and spaced in a 2 × 4 grid with 12 cm spacing between flowers, in the centre of the cage. Flower colours were arranged in a systematic alternating design (Fig. 1). In these conditions, butterflies readily fed, mated and laid eggs, and lived on average 12.4 days.

Butterflies were released into the cage immediately after emergence, and subjected to a sequence of three conditioning periods each of 3 days duration. At the end of each conditioning period butterfly behaviour was scored for 4 h.

Conditioning period 1. During this 3 day period, nectar was available in both red and blue flowers, so that all flowers provided an equally good source of nectar. At the end of this period we scored the number of visits to each flower colour, to establish whether the butterflies favoured a particular flower colour when nectar was equally available in both. Visits were only recorded if the butterfly landed

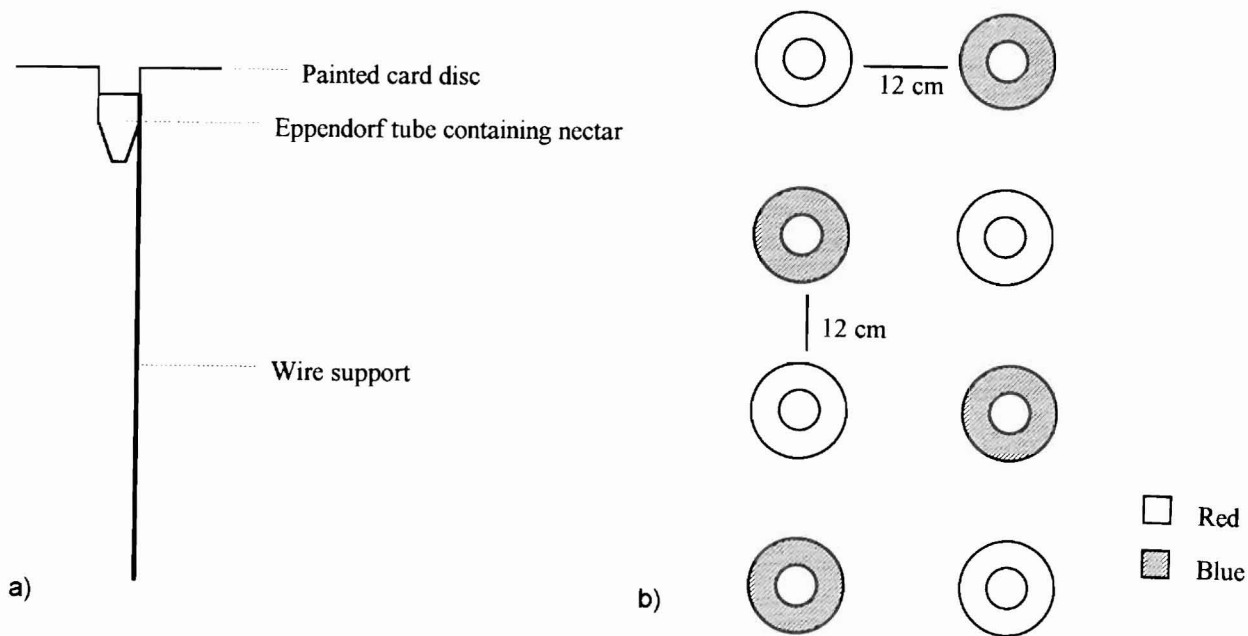


Fig. 1. (a) Construction and (b) arrangement of artificial flowers. Dimensions in the text.

on the flower and uncoiled its proboscis. At the end of every hour during the 4 h of scoring, the position of red and blue flowers in the grid was reversed, to allow for spatial preferences in foraging patterns.

To provide information as to how individuals responded to nectar availability in the two flower colours, butterflies were marked during scoring following conditioning period I. On their first visit to a flower the settled butterflies were picked from the flower and marked on the underside of the hindwing with a red or blue spot according to the colour of the flower they were feeding from, using an indelible ink marker pen. Subsequently a score was kept of the marked colour of butterflies against the colour of the flower they visited (excluding the first visit). Thus individual preferences for flower colour could be discerned, if present.

Conditioning period II. During the following 3 day period nectar was available from flowers of only one colour, to examine whether butterflies were able to learn to favour flowers which provide a reward in preference to those which provided none. At the end of this conditioning period visits to each flower colour were scored as above. Immediately prior to scoring, nectar was placed in all flowers, so that any preference could not result from butterflies using visual or olfactory clues to discern the nectar content of flowers (the possibility that butterflies marked flowers with pheromones we considered to be most unlikely).

Conditioning period III. During the final 3 day conditioning period, nectar availability was switched so that the flower colour which contained nectar during period II was empty, and vice versa, to examine whether learned preferences, if present, could subsequently be modified. Once again, immediately prior to scoring, nectar was placed in all flowers, and visits to flowers recorded as above.

Throughout the 9 days of the experiment, flowers were washed and filled daily with nectar if appropriate.

To provide replication the entire experiment was repeated on a second generation of butterflies. In the first generation eighty-two butterflies were used (forty-two female and forty male), in the second eighty-five (forty-six female and thirty-nine male). For the first generation nectar was placed in red flowers only during conditioning period II, while for the second generation nectar was placed in blue flowers only during conditioning period II.

Statistical analysis

The numbers of visits to each flower colour after each conditioning period were tested for approximation to the normal distribution by pooling deviations from the mean for each batch of four replicates in each period, and using a G-test for deviation from expectation (Sokal & Rohlf, 1981). The data were then analysed using a multi-factorial analysis of variance using colour, conditioning, replicate (hours 1–4) and generation as factors, with all pairwise interactions. Non-significant factors were removed in a stepwise manner. Significant factors were further inves-

tigated by *t*-test (assuming heteroscedasticity) for differences between the number of butterflies visiting red or blue flowers.

χ^2 tests were used to analyse whether the marked colour of an individual insect corresponded to the colour of flowers it visited. For example, did insects which had first been recorded on red flowers tend, on subsequent visits, to visit red flowers, or were visits random with respect to their first visit? Thus the proportion of insects feeding on the same colour as their mark was compared to the number of insects feeding on the different colour.

Results

The number of butterflies visiting each flower colour after each conditioning period is shown in Table 1. Numbers varied significantly between conditioning treatments ($F_{3,42} = 3.63$, $P = 0.035$). Colour alone had no significant effect, approximately equal numbers of butterflies visiting flowers of each colour when averaged over all conditioning treatments ($F_{1,46} = 0.03$, $P = 0.863$), but the conditioning treatment \times colour interaction was significant ($F_{3,42} = 103.52$, $P < 0.0001$).

t-tests for differences between the number of butterflies visiting red or blue flowers after each conditioning period were carried out to investigate causes of the significant treatment \times colour interaction (Table 1). When both flower colours contain nectar (period I) no significant preference was exhibited for one colour or the other. After 3 days during which nectar was available from only one of the two flower colours there was always a highly significant preference for the colour which contained nectar during conditioning. This preference cannot be due to an ability to distinguish between full and empty flowers on the basis of visual or olfactory cues, as during the 4 h of recording both flower colours were filled with nectar. The preference readily changed according to which colour had contained nectar most recently.

Individual preferences for colour were immediately apparent from casual observations of butterflies in the flight cage. Butterflies frequently fluttered around flowers prior to alighting on them, often fluttering above several flowers before settling. When this occurred, individuals usually fluttered above flowers of the same colour, showing no response to flowers of the opposite colour even when passing close to them. This observation is supported by the colour of flowers visited by marked butterflies (Table 2). Following conditioning period I, the vast majority of butterflies consistently visited the same flower colour on all foraging bouts: of 296 visits by marked butterflies to flowers, 271 were to the same flower colour as that first visited (92%), and only twenty-five to the opposite colour (generations 1 and 2 combined). This is a significant departure from the null hypothesis of no preference ($\chi^2 = 215.0$, $df = 31$, $P < 0.001$). As butterflies were not individually marked, we cannot say precisely what proportion were strictly constant. However, a minimum estimate can be calculated by assuming that all twenty-five infidelities

Table 1. Number of visits per hour to each of the flower colours, red and blue (individual flowers combined), following conditioning periods I–III, replicated in two successive generations. Numbers given are means for four consecutive hours of recording. *t*-tests were carried out for differences between the means.

Conditioning period	Generation	Blue		Red		<i>t</i> and <i>P</i>
		Mean (<i>n</i> = 4)	σ	Mean (<i>n</i> = 4)	σ	
I: nectar in both flower colours	1	56.0	9.5	57.8	6.2	<i>t</i> = 0.27, <i>P</i> = 0.801
	2	58.0	5.1	59.5	6.8	<i>t</i> = 0.30, <i>P</i> = 0.773
II: nectar in blue (generation 1), or red (generation 2)	1	80.3	9.4	35.5	4.6	<i>t</i> = 7.39, <i>P</i> = 0.002
	2	28.0	6.7	73.5	17.4	<i>t</i> = 4.87, <i>P</i> = 0.017
III: nectar in red (generation 1), or blue (generation 2)	1	26.3	9.8	70.5	7.0	<i>t</i> = 6.37, <i>P</i> < 0.001
	2	79.8	6.7	28.8	10.9	<i>t</i> = 7.99, <i>P</i> < 0.001

Table 2. Flower colour preferences of marked butterflies. Butterflies were marked according to the colour of the first flower visited, and subsequent choice of flower colour scored (the first visit is not included). Figures indicate the total number of visits by all butterflies in 1 h, arranged according to the colour of their mark and the colour of the flower visited. Means are calculated from four 1 h replicates. Figures in bold indicate butterflies visiting the same flower colour as their mark.

Conditioning period	Generation	Flower colour visited:				Colour of mark on butterfly:			
		Blue		Red		Blue		Red	
		Mean	σ	Mean	σ	Mean	σ	Mean	σ
I	1	18.3	10.5	1.3	0.8	2.3	1.1	15.3	6.4
	2	16.5	6.7	0.8	0.8	2.0	1.2	17.5	8.6
II	1	40.5	3.6	18.0	2.5	2.8	1.3	24.0	2.5
	2	15.3	3.5	2.3	1.6	18.8	2.9	44.3	9.4
III	1	17.0	2.7	5.3	3.0	12.3	1.9	41.5	6.7
	2	35.5	5.3	17.8	5.1	2.0	0.7	21.5	5.3

were made by different individuals, leaving 85% of individuals strictly constant during the observation period.

Following conditioning period II, in which nectar was available in only one flower colour, an increased number of butterflies began feeding at the flower colour which they had previously avoided. For example, in generation 1, when nectar was available only from blue flowers, many butterflies which prior to conditioning had fed predominantly on red flowers switched to feeding on blue. However, many red marked individuals persisted in visiting red flowers even when these had remained empty for 3 days (57%: 96/168 visits).

These patterns were consistent following conditioning periods II and III for both generations of butterflies. When nectar was available from only one flower colour, butterflies marked with that colour were almost invariably faithful to the colour (93%: 647/696 visits). Butterflies

marked with the other colour tended to switch preference, less than half of all visits being to their marked colour (46%: 267/578 visits). This difference is significant ($\chi^2 = 288.7$, *df* = 63, *P* < 0.001). Despite this, a significant trend remains towards individuals feeding at the flower colour they were originally marked on, even 6 days after marking ($\chi^2 = 538.9$, *df* = 63, *P* < 0.0001, periods II and III combined; $\chi^2 = 293.8$, *df* = 31, *P* < 0.001, period II; $\chi^2 = 245.1$, *df* = 31, *P* < 0.001, period III).

Discussion

When given a choice of (artificial) flowers of equal nectar content, but differing in colour, adult *P. napi* showed a marked flower constancy, in agreement with the findings of Lewis (1989) for *P. rapae*. Approximately equal numbers

of butterflies preferred red flowers to blue, suggesting that the butterflies had no inherent preference for one particular colour. The high fidelity described suggests that some individuals never sampled the other colour, and hence had no information as to the reward it provided.

The lack of an overall preference for red or blue in the absence of any difference in reward is perhaps surprising as red is not detected by many insect eyes. However, red-absorbing pigments were found in nine of seventeen lepidopteran species studied by Bernard (1979), while the butterfly *Pieris brassicae* shows a preference for red, yellow or blue-violet flowers (Barth, 1985).

The observed behavioural plasticity which enables butterflies to switch flower preference following conditioning is similar to that described in bees (Menzel, 1967; Wells & Wells, 1986; Wells *et al.*, 1992; Giurfa & Núñez, 1992). This is not surprising, since both are utilizing the same resource, but refutes the suggestion that the ability to learn foraging preferences is related to the complex social organization of bees (Menzel, 1984). Flexible foraging preferences are clearly of adaptive value, for an insect which is incapable of learning by experience will spend time and expend energy repeatedly visiting flowers which provide a low reward or which have a structure unsuitable for its mouthparts. Alternatively, a nectarivore with an inflexible preference for a particular flower will be unable to adapt to temporal or geographic variation in availability of flower species (Waser, 1978). Flowering in plants is often highly seasonal, and the distribution of nectar resources may switch markedly within the lifetime of a butterfly, so that a plant species which may be an excellent nectar source at the time of emergence may have finished flowering a week or two later, while during this period other plants may have come into flower.

The only behaviour described here which is inconsistent with that found in bees is the degree to which butterflies persist in visiting flowers which are empty: after conditioning away from their preferred flower colour for 3 days, 46% of visits were still to the favoured colour. Strict constancy despite a superior reward from alternative flowers has been described in bees (Wells *et al.*, 1992), but never to our knowledge have bees been found to persist in visiting empty flowers to a comparable extent. This frequency of visits is not plausibly explained by 'minoring': monitoring the rewards provided by non-preferred flowers by occasional infidelity (Heinrich, 1976). Butterflies may simply be slower in adapting their foraging preferences: switching to a different flower necessitates a temporary increase in handling time as the insect learns how to locate nectar (Laverty & Plowright, 1988; Lewis, 1986, 1993). Switching also incurs the cost of impaired ability to handle the original flower species (Waser, 1986). Both butterflies (Stanton, 1984; Lewis, 1986) and bees (Heinrich *et al.*, 1977; Gould, 1985) have a limited memory: knowledge of how to manipulate one flower type can be lost simply because it is replaced by information about a new flower type. As memory capacity decreases so constancy is favoured (Waser, 1986). Hence persistent preferences may be explained by a lower capacity for learning compared to

bees. Waser (1986) argues that the foraging behaviour adopted by a particular insect is determined by the limitations of its nervous system. Clearly more light may be shed on the factors favouring flower constancy if studies of nectarivore behaviour are expanded to include insect groups other than Hymenoptera.

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