

Corrigendum

Goyret, J. and Raguso, R. A. (2006). The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. *J. Exp. Biol.* **209**, 1585-1593.

The legend to Fig. 1 was published incorrectly in both the print and on-line versions of the article. The correct legend should read:

Fig. 1. Five different two-dimensional flower morphs tested in Experiment 1. (A) ‘No transparency’ paper flowers, whose surfaces are covered with acetate film cut to their exact shape, to control for fine texture. (B) ‘Transparency’ flowers covered with a square (9 cm×9 cm) sheet of acetate film. Arrows and brackets indicate *a priori* comparisons: (I) ‘Half lobes vs medium disks’ compares flowers with similar surface area but different edge-to-center distances. (II) ‘Half lobes vs small disks’ – compares flowers that have different surface area but the same edge-to-center distances. Note: all flowers have accessible nectaries at their centers.

The authors apologise for this error and any inconvenience caused.

The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*

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Accepted 13 February 2006

Summary

Nectar-foraging animals are known to utilize nectar guides – patterns of visual contrast in flowers – to find hidden nectar. However, few studies have explored the potential for mechanosensory cues to function as nectar guides, particularly for nocturnal pollinators such as the tobacco hornworm moth, *Manduca sexta*. We used arrays of artificial flowers to investigate the flower handling behavior (the ability to locate and drink from floral nectaries) of naïve moths, looking specifically at: (1) how the shape and size of flat (two-dimensional) artificial corollas affect nectar discovery and (2) whether three-dimensional features of the corolla can be used to facilitate nectar discovery. In these experiments, we decoupled visual from tactile flower features to explore the role of mechanosensory input, putatively attained *via* the extended proboscides of hovering moths. In addition, we examined changes in nectar discovery times within single

foraging bouts to test whether moths can learn to handle different kinds of artificial flowers. We found that corolla surface area negatively affects flower handling efficiency, and that reliable mechanosensory input is crucial for the moths' performance. We also found that three-dimensional features of the corolla, such as grooves, can significantly affect the foraging behavior, both positively (when grooves converge to the nectary) and negatively (when grooves are unnaturally oriented). Lastly, we observed that moths can decrease nectar discovery time during a single foraging bout. This apparent learning ability seems to be possible only when reliable mechanosensory input is available.

Key words: pollination, Lepidoptera, sensory, multimodal, Sphingidae.

Introduction

One of the 'mysteries of nature' revealed by Sprengel's landmark (Sprengel, 1793) publication was the concept of nectar guides – visually contrasting markings or aspects of flower morphology – that indicate the location of nectar to animal pollinators. The ubiquity of such markings, particularly those perceived in ultraviolet (UV) wavelengths, is one of the primary arguments made for the importance of contrasting flower colors to the visual perception and foraging behavior of insect pollinators (Menzel and Schmida, 1993; Chittka et al., 1994; Lunau et al., 1996). For example, honeybees show an innate proboscis extension reflex (PER) to UV marks at the center of *Helianthus rigidus* sunflowers, and probe at the periphery of the flower when the orientation of the ray florets is reversed (Daumer, 1958). However, it is unlikely that vision is the only sensory modality used by animals to find the nectar within flowers. Kevan and Lane (Kevan and Lane, 1985) showed that honeybees can detect differences in petal surface cell texture, and can learn such differences in conjunction with nectar rewards. Thus, tactile floral cues also could function as

nectar guides (Leppik, 1956; Glover and Martin, 1998), especially for animals with poor vision, or those that forage under low light conditions, such as crepuscular or nocturnal hawkmoths (Lepidoptera: Sphingidae).

Hawkmoths are abundant in tropical and warm-temperate habitats worldwide, where they constitute an important class of pollinators (Grant, 1983; Nilsson et al., 1987; Haber and Frankie, 1989). Olfactory and visual floral stimuli are known to attract several species within an appetitive context (Knoll, 1926; Kugler, 1971; Haber, 1984; Kelber, 1997). The European *Deilephila elpenor* and *Macroglossum stellatarum* utilize true color vision even under starlit conditions (Kelber and Hénique, 1999; Kelber et al., 2002), and modify their innate odor and color preferences through associative learning (Kelber, 1996; Balkenius and Kelber, 2004). *Manduca sexta*, a large nocturnal hawkmoth native to the Americas, also can learn particular odors associated with nectar rewards (Daly and Smith, 2000; Daly et al., 2001a). The blue photoreceptors have been identified as the major visual mediators of feeding behavior in *M. sexta* (Cutler et al., 1995), whereas ultraviolet

wavelengths were found to inhibit its feeding response (White et al., 1994). Floral odors attract *M. sexta* from a distance (3 m) in wind tunnel assays (Raguso and Willis, 2003; Raguso et al., 2005), and synergize with visual cues to activate feeding behavior (i.e. proboscis extension while hovering) in both naïve and wild moths (Raguso and Willis, 2002; Raguso and Willis, 2005).

However, successful approach to floral nectar sources and release of feeding behavior must be followed by reliable nectar assessment of individual flowers. Locating the nectary within a flower (evaluating the energy resource) is as critical as searching efficiently in order to find that flower. The hovering flight of *M. sexta* is an energetically expensive activity (Heinrich, 1971; Ziegler and Schulz, 1986), thus, the efficiency with which these moths handle flowers should be subject to selective pressures. *Manduca sexta* has a broad geographical distribution with several generations per year and it visits a wide variety of flower types across its range (Fleming, 1970; Raguso et al., 2003; Nattero et al., 2003). These observations led us to ask whether *M. sexta* can handle some flower morphologies more easily than others, and whether they can learn to handle flowers more efficiently with time. Such abilities would be consistent with their generalist foraging behavior and would allow these moths to efficiently assess flower profitability, as do other generalist flower visiting insects, such as bumblebees (Lavery and Plowright, 1988; Chittka and Thomson, 1997) and *Pieris* butterflies (Lewis, 1986).

The question remains as to which sensory modalities adult *M. sexta* might utilize for such a task. The diurnal hawkmoth *Macroglossum stellatarum* utilizes contrasting marks on the surface of flower corollas by preferentially placing its proboscis on such visual nectar guides (Knoll, 1922). Thus, *M. stellatarum* uses visual cues not only while searching (in flight) for nectar sources (Kelber, 1997), but also while hovering a relatively short distance (proboscis length: 2.5 cm) in front of individual flowers. Owing to its long (8–10 cm) tongue, *M. sexta* also hovers at a distance from flowers while feeding, such that in most cases, its only physical contact with flowers is through the proboscis. Here we ask whether mechanosensory input to the proboscis is redundant or complementary to the visual stimuli used by *M. sexta* when freely foraging on artificial flowers. In the first experiment, we decoupled visual from tactile stimuli by placing flat square transparency film sheets over the corolla portion of plain-white artificial flowers to test whether these moths use mechanosensory stimuli to find nectar within individual flowers. If visual stimuli are sufficient, hawkmoths should show comparable handling efficiencies on the same flower models, whether or not they are covered with transparency film. We repeated this comparison among five different artificial flower morphologies, systematically varying corolla shape and surface area.

In the second experiment, we tested whether groove-like folds, usually found in the corollas of flowers visited by hawkmoths, affect flower handling by *M. sexta*. We also evaluated flower handling performance in relation to different

artificial flower morphologies by comparing total, successful and unsuccessful visits of individual moths foraging on arrays of 12 flowers. Finally, we examined whether moths can learn to handle different flower morphs more efficiently within a single foraging bout by examining the time they took to find nectaries as foraging bouts progressed.

Materials and methods

Animal care

This study was carried out from September to December 2004 at the University of South Carolina, Columbia, SC, USA. We used 3- to 5-day-old *M. sexta* L. adults reared from eggs provided by Dr Lynn Riddiford, University of Washington, Seattle, WA, USA. Larvae were fed *ad libitum* on an artificial diet (Bell and Joachim, 1976) and were kept, as pupae, under a 16 h:8 h light:dark cycle (24:21°C), in a humidified atmosphere. Male and female pupae were kept in separate incubators (Precision 818, Winchester, VA, USA) under the same ambient regime and emerged within 45×45×45 cm screen cages (BioQuip, Inc., Rancho Dominguez, CA, USA). Adults were starved for 3–4 days before being used in experiments.

Experimental arena and flight assays

At the beginning of scotophase (15:00 h, temperature range: 22–25°C), naïve moths were placed individually within a closed Tedlar mesh flight enclosure (Bioquip; 2 m×2 m×2 m). The flight cage included an experimental floral array (20 cm×30 cm×45 cm) placed over a dark, odor-permeable box constructed by covering a matte-black-painted aluminum grid with black cheesecloth. To provide appropriate olfactory cues and humidity, we placed the cheesecloth-covered grid over two 200 ml glass beakers filled with water, each of which contained a cotton-tipped applicator swab impregnated with two drops of undiluted bergamot oil (Body Shop, Columbia, SC, USA). Thus, odor and water vapor passed through the cheese cloth and permeated the flight chamber. Bergamot oil is chemically similar to the odors of many hawkmoth-pollinated flowers (Kaiser, 1993; Knudsen and Tollsten, 1993; Mondello et al., 1998), and pilot experiments revealed it to be a potent releaser of feeding behavior in *M. sexta*. Visual floral stimuli were provided by a 3×4 array of artificial flowers (see below), in which each flower was separated from its neighbor by 10 cm. Artificial flowers were bathed in odor and water vapor that diffused freely through the cheesecloth. The flight enclosure was lit with a dim red light [wavelengths >600 nm (see Raguso and Willis, 2002)]. Each trial involved only one moth, which was allowed to fly freely. If the moth did not find, approach or probe the flowers within 5 min, it was captured and discarded. If it found the flowers, it was allowed to forage for a maximum of 10 min after the first floral approach. Foraging bouts were recorded with a video camera (Sony Digital 8 –TRV120 Best Buy, Columbia, SC, USA) in ‘night-shot’ mode placed outside the flight enclosure.

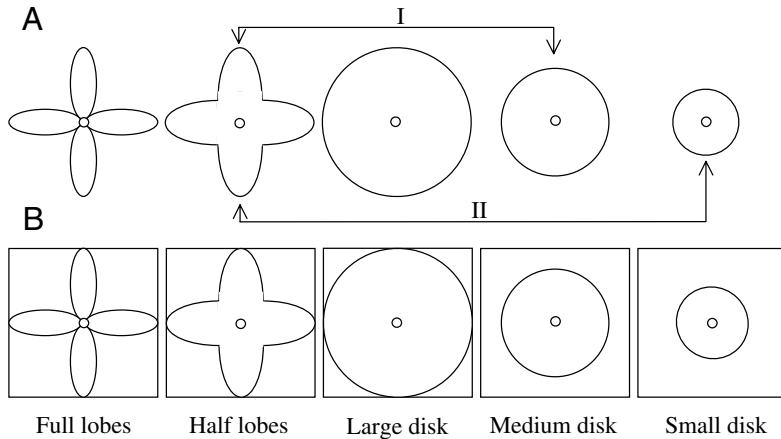


Fig. 1. Five different two-dimensional flower morphs tested in Experiment 1. (A) 'No transparency' paper flowers, whose surfaces are covered with acetate film cut to their exact shape, to control for fine texture. (B) 'Transparency' flowers covered with a square (9 cm \times 9 cm) sheet of acetate film. Arrows and brackets indicate *a priori* comparisons: (I) 'half lobes vs medium disks' compares flowers that have different surface area but the same edge-to-center distances. (II) 'Half lobes vs small disks' compares flowers with similar surface area but different edge-to-center distances. Note: all flowers have accessible nectaries at their centers.

Experiment 1

In each trial, individual moths were offered different homogeneous arrays (12 flowers of the same morph) displayed as described above. We used light-grey paper with low UV reflectance (Kinkos 'Grey fleck'; wavelength reflectance 80% of a barium sulfate 'white' standard above 420 nm, <50% below 400 nm) to construct five different flower morphs (Fig. 1A), as follows. Full lobes: four elliptical lobes or petals with a semi-major axis of 2.2 cm and a semi-minor axis of 0.8 cm each. Total area: 21.4 cm². Flower span: 9 cm. Half lobes: four elliptical lobes with a semi-major axis of 3.2 cm and a semi-minor axis of 1.3 cm each. Every lobe overlaps with the adjacent ones leaving a squared center with sides of 2.7 cm. Half of each ellipse appears as a petal. Total area, 33.7 cm²; flower span, 9 cm. Large disk: a disk with a diameter of 9 cm and an area of 63.6 cm². Medium disk: a disk with a diameter of 6.5 cm and an area of 33.2 cm². Small disk: a disk with a diameter of 4 cm and an area of 12.57 cm². Additionally, the corolla portion of each of the five flower morphs was covered with a square transparency film sheet (henceforth called transparency treatments; Fig. 1B), accounting for a total of 10 treatments. In this way, we could evaluate the foraging behavior under circumstances where no reliable tactile stimuli were available to the animals, but visual stimuli could be preserved. To control for any surface texture effect, flowers in the treatments lacking a transparency square above them (henceforth called no transparency treatments) were covered with transparency film that had been cut to match the exact shape of the underlying paper flower. Each flower from every treatment offered 20 μ l of a 20% (w/w) sucrose solution in a nectary (5 cm long by 0.5 cm opening diameter pipette tip) placed at its center; nectaries were accessible to moths through a 0.5 cm opening cut into the transparency film in both treatments. Each nectary was attached to the flower such that it did not protrude above the flower surface.

Variables recorded

We recorded the foraging efficiency (number of successfully exploited flowers over 10 min) after each trial. The number and duration of total, successful and unsuccessful flower visits

were recorded from video-tape playback and timed with a Mistral chronometer (Buenos Aires, Argentina) to a resolution of 1 s.

Each flower visit began at the moment the proboscis made contact with the flower. Unsuccessful visits ended when the proboscis lost contact with the artificial flower without having reached the nectary. Successful visits were recorded until the proboscis was inserted into the nectary; when drinking time was recorded as the time elapsed until the proboscis was removed. The ratio of successful to total visits (successful visits/total visits; where total visits = unsuccessful visits + successful visits) was established as an indicator of the animals' efficiency when foraging on the different flower morphs.

Given that we had recorded the time moths took visiting each flower, we tested whether moths could learn to handle the different flower morphs during a single foraging bout. Discovery time was defined by the time elapsed between the initiation of flower probing and the entry of the proboscis into the nectary. This does not include the time flying from one flower to another or drinking, but only the time spent probing at the flower's threshold. We measured discovery time for the first eight successful visits, as did Lewis (Lewis, 1986).

Experiment 2

A second experiment was carried out to evaluate whether *M. sexta* can use morphological features of flowers involving a third dimension (i.e. depth) to improve its foraging efficiency. Many night-blooming flowers (e.g. *Datura*, *Mirabilis*) have conspicuously grooved petals, which could in theory be used as tactile guides for the moths' proboscides (Fig. 6). Thus, three treatments were designed. The first was 'medium disks', the same flat flowers used in Experiment 1. The second and third were paper disks of the same diameter as medium disks, with two groove-like folds (see Fig. 2). In the second treatment, the folds were oriented parallel to each other ('chord grooves') and were placed 1.5 cm apart from the origin (nectary) of the disk (Fig. 2). In the third treatment, the folds were placed as two orthogonal diameters of the disk ('radial grooves'), intersecting at the nectary (Fig. 2).

Table 1. Variables recorded in relation to flower handling by *Manduca sexta* on different flower morphs with and without a square transparency film

Number of visits (mean)	No transparency film					Transparency film				
	FL(16)	HL(18)	LD(17)	MD(16)	SD(16)	FL(17)	HL(19)	LD(17)	MD(16)	SD(16)
Total	85.11±8.7	73.20±15.4	48.25±10.3	81.69±10.5	77.88±11.1	65.53±16.4	74.88±9.2	54.44±10	39.20±6.9	85.11±11.81
Successful	50.39±5.9	21.4±4.4	5.81±2.2	18.38±3.7	51.18±6	8.63±2.7	13.47±3.6	6.89±2.8	4.13±1.4	22.69±5.6
Failed	34.72±5.6	51.8±7.9	42.44±9.5	63.31±6.5	26.71±4.2	60.94±13.9	61.41±8.8	47.56±14.1	35.07±8.8	41.63±8.6
Ratio of successful/total	0.6±0.04	0.24±0.04	0.08±0.02	0.21±0.02	0.65±0.05	0.08±0.02	0.15±0.03	0.09±0.03	0.07±0.02	0.26±0.07

All values are means ± s.e.m. Numbers in parentheses are the number of replicates for each treatment. FL, full lobe; HL, half lobe; LD, large disc; MD, medium disc; SD, small disk.

Statistical analysis

Response levels of male and female *M. sexta* to different flower morphs in Experiment 1 were tested by means of log-likelihood tests (*G*-tests using the *G_h* test statistic). Foraging efficiency, measured with the variables, emptied flowers and ratio of successful/total visits, was tested with the Kruskal–Wallis non-parametric test using a corrected α -level of significance of 0.005. Thus, we performed ten statistical tests using the same set of data: six for emptied flowers, three for ratio of successful/total visits and one linear regression). Discovery time as a function of the sequence of feeding attempts was tested to fit the classic exponential decline learning curve described by Hilgard and Bower (Hilgard and Bower, 1966). A corrected α -level of significance of 0.008 was used in these tests (six regression analyses).

Because the variables measuring moths' foraging success on model flowers (emptied flowers and ratio of successful to total visits) showed equivalent results in Experiment 1 (see Results), we only analyzed emptied flowers data in Experiment 2. Two *a priori* comparisons were planned (control group, i.e. medium disks vs radial grooves, and control vs chord grooves). Our evaluation of emptied flowers and the appropriate contrasts were performed using one-way analysis of variance (ANOVA) and *t*-tests, respectively, because the assumptions of the model (normality and homogeneity of variances) were met.

Results

Experiment 1

Inside the flight cage, 71.4% of the experimental animals (*N*=172) approached and probed the artificial flowers, with no significant gender differences observed (females: 66.4%; males: 76.2%; *G_h*=1.81; *P*=0.6).

There were no differences in the overall proportions of responses to the different flower morphs, either with or without square transparency film (*G_h*=5.85; *P*=0.56; Table 1). Variation in flower shape and size did not account for any difference in initial feeding responses (i.e. approaches and probes). The presence of the square transparency film had a significant effect on the number of artificial flowers that moths successfully exploited ('emptied flowers') during each foraging bout (Kruskal–Wallis test; transparency vs no transparency: $\chi^2_{(1,0.005)}=18.43$; *P*<0.0001; Table 1, Fig. 3). In

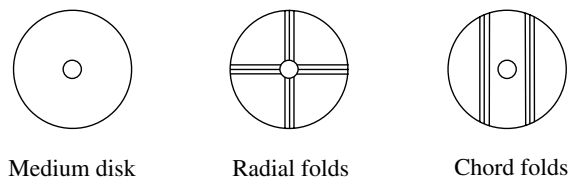


Fig. 2. Three-dimensional flower morphs tested in Experiment 2. Medium disk: same disk as in Fig. 1. Radial folds: medium disk with two groove-like folds along two perpendicular diameters of the disk. Chord folds: medium disk with two groove-like folds along two parallel chords, each 1.5 cm apart from the origin of the disk.

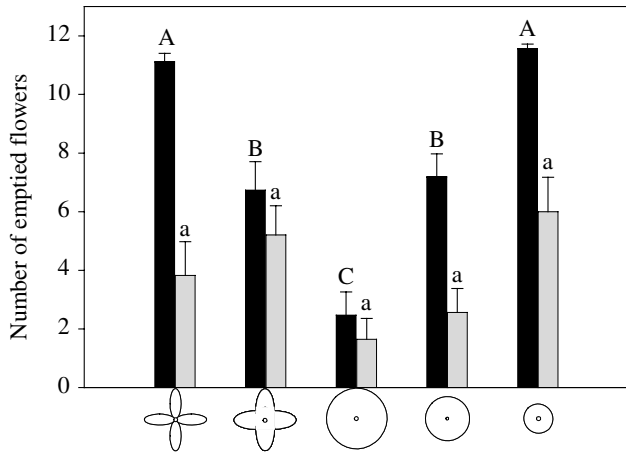


Fig. 3. Number of emptied flowers (foraging efficiency; mean \pm s.e.m.) after a 10 min foraging bout by individual *Manduca sexta* inside the flight chamber. In each treatment (abscissa) an array of 12 artificial flowers of the same morph was present. Black bars represent responses to artificial flowers without square transparency film; gray bars represent responses to the same artificial flowers covered with a square transparency film. Different letters denote statistically significant differences with a corrected α -level for significance of 0.008 (see text).

addition, variation in emptied flowers was significantly affected by flower morphology among the no transparency treatments (Kruskal–Wallis test; within no transparency: $\chi^2_{(4,0.005)}=44.64$; $P<0.0001$). This effect was not observed among transparency treatments; in this case differences were not as pronounced, only accounting for a trend (Kruskal–Wallis median test; within transparency: $\chi^2_{(4,0.005)}=10.18$; $P=0.04$).

Among the no transparency treatments, moths clearly were more successful when handling full lobe and small disk morphs (see Fig. 3). As flower surface area increased from small disk (12.6 cm²) to large disk (63.6 cm²), moth performance declined (see regression analysis below). This same effect was observed when comparing full lobe (21.4 cm²), half lobe (33.7 cm²) and large disk (Kruskal–Wallis test; full lobe vs half lobe: $\chi^2_{(1,0.005)}=6.07$; $P=0.014$; large disk vs medium disk: $\chi^2_{(1,0.005)}=16.63$; $P<0.0001$). Because surface area is not the only flower feature that varied among treatments, we tested whether the minimum distance from the edge to the nectary (center) of the flower could affect moths' performance independently. Half lobe and medium disk flowers have similar surface areas (33.7 cm² and 33.2 cm², respectively) but different edge-to-center distances (2 cm and 3.25 cm, respectively). Similarly, half lobe and small disk flowers have the same minimum edge-to-center distance (2 cm) but different surface areas (33.7 cm² and 12.6 cm², respectively). Surface area appeared to be a more important flower feature than edge-to-center distance, as feeding effectiveness did not differ significantly between half lobe and medium disk flowers (Kruskal–Wallis test; $\chi^2_{(1,0.005)}=0.005$; $P=0.945$), but differed significantly between half lobe and small disk flowers

(Kruskal–Wallis test; $\chi^2_{(1)}=18.34$; $P<0.0001$; α -level: 0.005). Moreover, the number of emptied flowers was significantly correlated with flower surface area ($a+\beta x=y$; $a=14.33$; $\beta=-0.75$; $R^2=0.56$; $F_{(1,0.005,84)}=105.5$; $P<0.0001$).

Analysis of the ratio of successful/total visits yielded the same results as obtained from the analysis of emptied flowers (Kruskal–Wallis tests; transparency vs no transparency: $\chi^2_{(1)}=30.48$; $P<0.0001$; within transparency: $\chi^2_{(4)}=59.29$; $P<0.0001$; within no transparency: $\chi^2_{(4,0.005)}=7.39$; $P=0.12$). Contrasts between flower morphs on this variable show the same significance levels as those on the emptied flowers variable.

Discovery times generally decreased when moths foraged on the artificial flowers (no transparency; exponential decline fit: $R^2=84.35$; $P=0.001$) as illustrated by Fig. 4A (full lobe with no transparency; exponential decline function fit: $R^2=84.87$; $P=0.001$). Nevertheless, this was not the case for the large disk treatment (Fig. 4C; exponential decline function fit: $R^2=37.99$; $P=0.10$). When flowers were covered by a transparency film, discovery times did not conform to a typical learning curve (transparency treatment; exponential decline function fit: $R^2=56.58$; $P=0.031$; corrected α -level of significance: 0.005), as shown in Fig. 4B,C (full lobe with transparency: $R^2=8.89$; $P=0.47$; large disk with transparency: $R^2=0.0$; $P=1.0$).

Experiment 2

Flower morphology was significantly associated with the number of emptied flowers when moths foraged on different flower arrays (ANOVA: $F_{(2,57)}=21.11$; $P<0.0001$). Both kinds of grooved artificial flowers affected the performance of foraging moths, but in opposite ways (Fig. 5). Moths performed worse on flowers with chord grooves than on flat control flowers (medium disk vs chord grooves: $F_{(1,39)}=78.9$; $P<0.0001$), whereas moth performance on flowers with radial grooves was significantly better than on flat control flowers (medium disk vs radial grooves: $F_{(1,37)}=328.11$; $P<0.0001$).

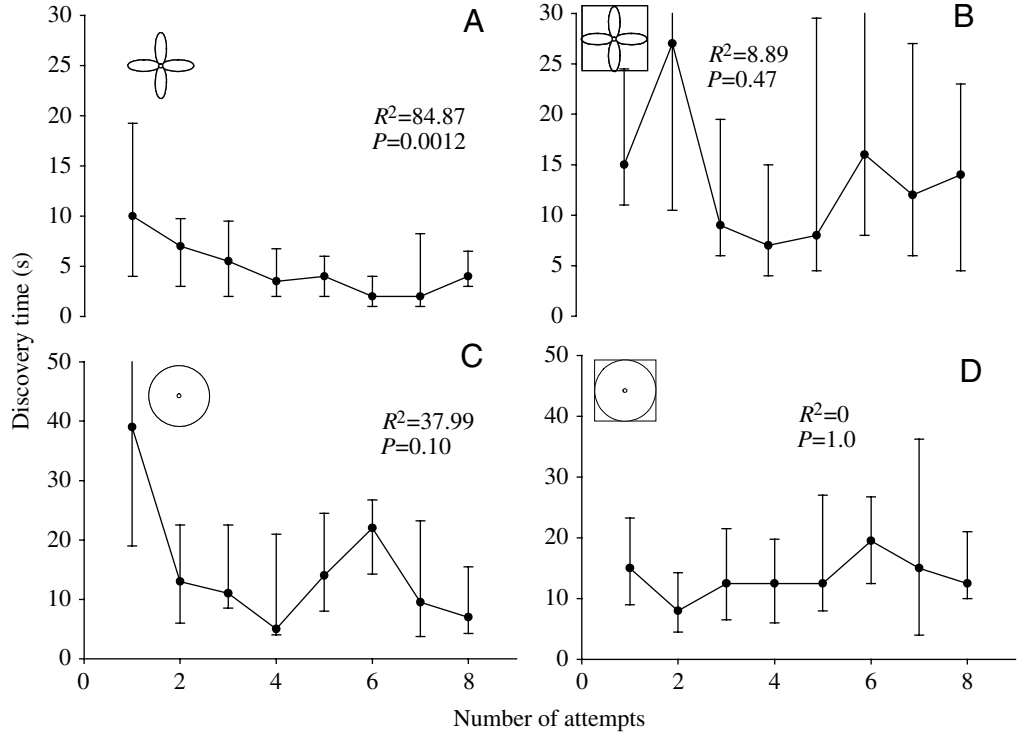
Discussion

Behavioral sequence of flower foraging and its distinct sensory modalities

The foraging behavior of *Manduca sexta* appears to follow a sequential pattern involving different sensory modalities at each stage (Raguso and Willis, 2003). Thus, a moth under appetitive motivation will first fly upwind when encountering an appropriate fragrance (Brantjes, 1978). At closer range, flower approach by *M. sexta* is guided by either olfactory or visual stimuli, whereas proboscis extension requires the combination of visual and olfactory cues (Raguso and Willis, 2002; Raguso and Willis, 2005). Here we show that mechanoreception is an additional sensory modality that contributes to the final stage of the feeding sequence, once the proboscis is extended and moths must locate and drink from floral nectaries, a process frequently referred to as 'flower handling'.

In our experiments, moths were effectively and equally

Fig. 4. Discovery time (probing time between feeding attempts) for four different treatments. (A) Full lobe, (B) full lobe with transparency film, (C) large disk and (D) large disk with transparency film. Data points are medians, whiskers represent first and third quartiles. Statistical values refer to goodness-of-fit to an exponential decline function (one factor), a classical ‘learning curve’. Moths exploiting full lobe flowers with no transparency film show exponentially decreasing discovery times. When exploiting large disks, or either shape with transparency film, moths show larger variances in their responses, which do not fit an exponential decline function.



attracted to the different artificial paper flowers, regardless of the fact that they differed in shape and size (which in turn, greatly affected performance), when paper flower arrays were presented with Bergamot oil as an olfactory stimulus (see Results). This result indicates that probing responses (i.e. emptied flowers) to different treatments were not confounded by innate differences in attractiveness, and that no biases in moth preference or attraction were associated with the

transparency films used to de-couple visual and mechanical stimuli.

Vision and mechanoreception during flower probing

What is the innate probing strategy of *M. sexta*? Is the proboscis guided visually or are there other sensory systems involved? The use of different artificial flowers affected the efficiency with which *M. sexta* foraged on them. The ‘lobes’ series and the ‘disks’ series (both of which include the large disk treatment) showed improvements in moth performance correlated with decreased surface area. As surface area increases, edge-to-center distance also increases, but the *a priori* comparisons (Figs 1, 3) strongly suggest that for the set of artificial flowers used in this study, surface area was the main corolla feature affecting performance. Furthermore, this hypothesis is supported by the significant linear regression between surface area and performance (i.e. emptied flowers). On flat disk flowers with no surface features, probing by naïve *M. sexta* is ineffectual on larger disks, as the moths probe across the disk’s surface and rarely find the centrally located nectary. Similarly, Knoll (Knoll, 1926) showed that when *Hyles lineata livornica* hawkmoths forage on artificial flowers, they probe the entire surface of the paper models. Our findings suggest that the innate strategy of *M. sexta* is to perform a ‘random walk’ of probing across the flower’s surface.

The disruption of reliable tactile information clearly interferes with flower handling by *M. sexta*, showing that mechanoreception, in addition to vision and olfaction, is involved in nectar feeding by these moths (Fig. 3). Tactile cues constitute an important component of many flower–pollinator systems (Kevan and Lane, 1985; Borg-Karlson, 1990), but are

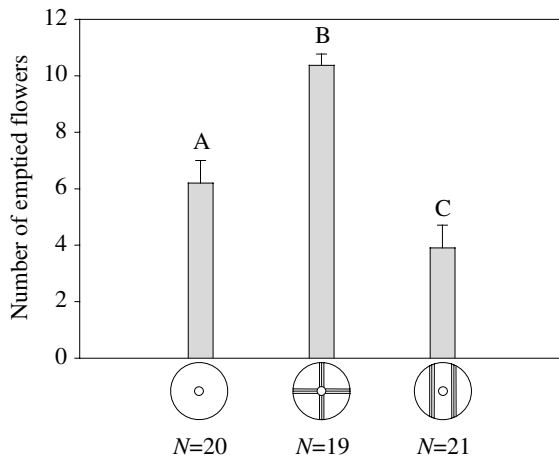


Fig. 5. Three-dimensional corolla features affect foraging efficiency by *Manduca sexta*. The vertical bars represent number of flowers (mean ± s.e.m.) emptied after a 10 min foraging bout by individual moths inside the flight chamber. In each treatment (abscissa) an array of 12 artificial flowers of the same morph was present. Different letters denote statistically significant differences (see text).

rarely investigated from a behavioral standpoint. Interestingly, in the treatments with transparency film, we observed an overall reduced performance to the point where variation in flower shape had no significant effect on handling efficiency. Moths performed equally poorly on the different flower models without reliable tactile information, despite the fact that visual differences were preserved. Further investigation of the influence of mechanoreception on probing behavior led to Experiment 2, in which we found that corolla grooves positively affect the handling performance when they converge at the nectary and negatively affect it when they are incorrectly oriented (Fig. 5). This suggests that three-dimensional features have a hierarchical precedence on nectar-searching behavior at the flower handling scale as proposed by Brantjes and Bos (Brantjes and Bos, 1980). At this level, the spatial resolution of *M. sexta*'s eyes does not allow for accurate feedback about proboscis position (A. Kelber, personal communication). The low signal-to-noise ratio of the visual modality at this scale could have imposed selective pressures for *M. sexta* to efficiently assess floral nectar content by other means. Such means include mechanoreception, as suggested by Leppik (Leppik, 1956) for some butterflies and as showed in this study, and probably gustation, given the responses of chemoreceptive sensilla positioned along the tip of the lepidopteran proboscis (Krenn, 1998; Kelber, 2003).

Spengel (Spengel, 1793) introduced the concept of nectar guides as floral features that could be used by pollinators to visually locate the nectar. Subsequent experiments revealed that the diurnal hawkmoth *Macroglossum stellatarum* (Knoll, 1922), bumblebees (Manning, 1956; Kugler, 1966), honeybees (Daumer, 1958; Free, 1979) and bee-flies (Johnson and Dafni, 1998), among other insects, successfully utilize visual nectar guides. Here we show that the utility of Spengel's idea extends beyond the visual system, as the tactile sensitivity of the proboscis of *M. sexta* allows these moths to exploit the physical features of flowers in order to find nectar (Figs 3, 5, 6). Our experiments, unlike those of Knoll (Knoll, 1922; Knoll, 1926), varied the contours of artificial flowers, rather than testing moth responses to natural flowers. Further experiments will be required to test whether visual nectar guides of color contrast can be used by *M. sexta*.

Context dependence of the floral visual display

This study indicates that once moths approach a flower patch, they extend their proboscides towards a visual target and then appear to rely on mechanosensory input. At this point, when probing is relatively random, any irregularity on the corolla surface could guide moths' searching behavior, such that the proboscis 'rides' along the length of petal grooves, nectary openings or the margins of highly divided corollas.

The funnel-shaped flowers of *Datura wrightii*, a favored nectar source of *M. sexta* in the Sonoran Desert (Raguso et al., 2003) are comparable in diameter to the large disk models in Experiment 1, but previous experiments indicate that *Datura* flowers are learned very quickly by naïve *M. sexta* (Desai and Raguso, 2001), which is not the case when foraging on our large



Fig. 6. Features of flower handling are illustrated in this photo of *Manduca sexta* feeding from a flower of *Mirabilis multiflora* (Nyctaginaceae). Note the extended proboscis (grey arrow), the distance of the moth's body from the flower, and the radial grooves in the flower's perianth (white arrows). Scale bar, 1 cm. Photo[©] Robert A. Raguso.

disks (Fig. 3). It appears that the decrement in flower handling by *M. sexta* on flowers with high surface area is offset by floral depth. However, attraction from a distance is enhanced by the increased visual display provided by flowers with larger diameters (Knoll, 1922). Tubular flowers appear to offer a compromise solution to this hypothetical trade-off, while simultaneously providing for high nectar volumes and appropriate physical contact between the body of the moth and the sexual organs of the flower (Nilsson, 1988). It is tempting to consider how differences in handling efficiency associated with corolla form might impact competition between night blooming flowers for hawkmoths as pollinators (see Haber and Frankie, 1989), however, most flowers in nature are likely to be visited by experienced moths. Additional experiments will be needed to determine whether the handling differences identified in this study have an impact on subsequent foraging decisions.

Flower handling improves with experience

We analyzed whether *M. sexta* could learn to improve its handling abilities (i.e. reduce the time to find nectar) during a single foraging bout. Indeed, *M. sexta* adults improve their handling of artificial flowers within an extended feeding bout (Fig. 4). We analyzed improvement in flower handling overall (with and without transparency film) and within two specific treatments – full lobe and large disk – as examples of flowers that elicited high and low performances, respectively. Flower handling did not improve on model flowers in which the nectary was difficult to find (large circles), nor when square transparency films prevented the acquisition of reliable mechanosensory information (see Fig. 4B,D). This suggests that reliable tactile information is needed not only to forage efficiently (Fig. 3), but also to learn to forage more efficiently (slope of learning curves, see Results and Fig. 4).

Learned improvement in flower handling has been shown in

a variety of nectivorous insects, including other lepidopterans (Lewis, 1986; Hartlieb, 1996; Cunningham et al., 1998) and hymenopterans (Harder, 1983; Laverty and Plowright, 1988; Chittka and Thomson, 1997). This ability gives animals the opportunity to decrease the time they spend on individual flowers and thus, directly increase their foraging efficiency and caloric intake (Pyke et al., 1977; Hughes and Seed, 1981). Learned flower handling (and its attendant constraints, i.e. the inability to learn more than one or a few floral species) has been hypothesized to account for facultative flower specialization through the advantage that generalist pollinators gain by learning to handle a particular floral species (Darwin, 1895; Lewis, 1986). This is supported by Lewis' (Lewis, 1986) observation that *Pieris rapae* butterflies trained to one flower type find it more difficult (than do naïve butterflies) to learn a second flower type. Moreover, bumblebees can associate the morphology of artificial flowers with their color (Chittka and Thomson, 1997). On natural flowers, preference for flowers that are more easily handled is shown by the specialist bumble bee *Bombus consobrinus* (Laverty and Plowright, 1988), and by two species of hummingbirds and bumblebees for blue-colored (over albino) flowers of *Delphinium nelsonii* (Waser and Price, 1983).

We have shown that naïve *Manduca sexta* hawkmoths are equally likely to feed from several different homogeneous arrays of artificial flowers with different morphologies. However, we did not explicitly test whether the moths have innate preferences for flower morphology in a dual choice setting, nor whether they develop preferences for different flower models *after* learning to handle them. Future studies should test whether naïve *M. sexta* prefer specific flower morphologies when faced with a mixed array, and if so, whether such preferences can be modified through experience.

We would like to thank Glenn Svensson and members of John Hildebrand's laboratory for fruitful discussions, David Wethey for statistical advice, and Richard Vogt and Addie Williams for help with moth culture. We also thank Michael Hickman and Anna Claire Vaughn for help with the flower models, initial data recording and analysis. Thanks to Valentin Bârca, Sheetal Desai and Melissa Jurkiewicz for the insights gained through their student projects. This study was funded by NSF grant IOB-0444163 to R.A.R.

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