Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*

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Communication between flowering plants and their pollinators involves innate, learned and deceptive sensory signals. Nocturnal, hawkmoth-pollinated flowers are among the most reflective and strongly scented known, suggesting the potential for multimodal sensory communication with pollinators. We measured the relative importance of these overstated signals, both alone and in combination, to nectar feeding by naïve male hawkmoths, *Manduca sexta*. In the absence of fragrance, moths hovered and repeatedly passed (in flight) within 1 m above arrays of model paper flowers. The presence of plant odours elicited significantly more hovers and close passes, as well as three additional behaviours: approaches to the paper flowers, approaches to the hidden odour source and nectar feeding. However, approaches to odour sources never resulted in proboscis extension or probing in the absence of a visual target. Nectar feeding increased six-fold when paper and natural flowers were presented in mixed arrays, but moths did not show preferential visits or constancy to either type of flower. Significantly ordered behavioural sequences were observed when both visual and olfactory stimuli were present, but not when fragrance was absent. The largest conditional probabilities indicated a chain of responses beginning with hovering and leading to floral approach and nectar feeding. As single modality cues, both floral fragrance and visual display are innately attractive to male *M. sexta*, but the combination thereof is required to elicit proboscis extension and feeding.

Communication in nature frequently requires the detection and integration of multimodal sensory signals. For example, elephants signal a state of musth using infrasonic, olfactory and visual signals that function in complementary or redundant ways, depending upon distance (Poole 1999; Rasmussen & Krishnamurthy 2000). In contrast, domestic chicks respond to the combination of pyrazine odour and red or yellow coloration with novel, aversive behaviours not elicited by either signal alone (Rowe & Guilford 1996). Finally, visual cues provide contextual information when paired with olfactory stimuli during classical conditioning of honeybees (Couvillon & Bitterman 1988; Gerber & Smith 1998).

Flowering plants use combinations of sensory signals (visual, olfactory, tactile, gustatory, even acoustic cues) to communicate with flower-visiting animals (Kevan & Baker 1983; Dobson 1994; Lunau & Maier 1995; von

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Helversen & von Helversen 1999). These signals indicate how to find flowers from a distance (Janzen 1971; Gottsberger & Silberbauer-Gottsberger 1991), whether a flower has already been visited (Lex 1954; Weiss 1995), and where to probe for hidden rewards (Waser & Price 1985; Lunau 1992). Pollinators that respond by visiting flowers more frequently, foraging more efficiently and remaining constant to that species provide quality reproductive services for plants and a mechanism for directional selection on flower morphology (Pyke 1978; Herrera 1987; Laverty & Plowright 1988). Diverse classes of flower-feeding animals demonstrate a broad spectrum of innate and learned responses to floral cues (Williams 1983; Weiss 2001). However, relatively few studies focus explicitly on interactions between the components of multimodal floral signals, whether at different spatial scales or different stages of pollinator experience (Borg-Karlson 1990; Raguso 2001).

We investigated the importance of visual and olfactory floral signals to nectar foraging by nocturnal hawkmoths (Sphingidae: Lepidoptera), a distinctive class of animal pollinators. Like hummingbirds, hawkmoths use nectar to fuel metabolically expensive hovering (Heinrich &

Table 1. A priori hypotheses and predictions for the function of scent and visual cues in hawkmoth attraction and feeding

Critical predictions	Hypotheses				Experimental results (this study)
	1a (visual only)	1b (scent only)	2a (visual attraction/ scent feed)	2b (scent attraction/ visual feed)	
Scent is required to initiate feeding	No	Yes	Yes	Yes	Yes
Scent is required to feed at each flower	No	Yes	Yes	No	No
Visual cues are required for feeding	Yes	No	Yes	Yes	Yes

Casey 1973; Casey 1976; Bartholomew & Casey 1978) and long-distance flights in search of mates and appropriate host plants (Cross & Owen 1970; Janzen 1986, 1987; Powell & Brown 1990). While hawkmoths forage opportunistically on a variety of flowers (Kislev et al. 1972; Haber & Frankie 1989), they effectively pollinate large, tubular night-blooming flowers with bright, reflective coloration and powerful fragrances (Gregory 1964; Grant 1983; Nilsson et al. 1987; Willmott & Búrquez 1996). In comparison to diurnal flowers, the striking visual displays and perfumes of these flowers constitute supernormal stimuli (Haber & Frankie 1989; Thompson 1994), at the cost of attracting unbidden herbivores and seed predators (Baldwin et al. 1997). It has long been assumed that such overstated floral advertisement has evolved to increase the likelihood of hawkmoth attraction and pollen movement among populations of such plants (Baerends 1950; Baker 1961; Brantjes 1978). To what extent do nocturnal hawkmoths use visual and olfactory cues to find flowers and feed from them? We addressed this question using the tobacco hornworm moth, *Manduca sexta*, which is representative of a large guild of nocturnal hawkmoths distributed across warm-temperate and tropical America (Grant 1983; Janzen 1986). Although there is a sizable body of research on *M. sexta*'s visual and olfactory physiology (White et al. 1983; Christensen et al. 1993; Hildebrand 1996), balanced experiments testing the importance of each modality in feeding behaviour are lacking.

The large compound eyes of *M. sexta* have three visual pigments with absorption maxima at 360 (UV), 450 (blue) and 560 nm (green-yellow) (Bennett & White 1989; Bennett et al. 1997). Laboratory-reared *M. sexta* show wavelength-specific feeding behaviour, visiting artificial flowers that transmit blue and/or green-yellow wavelengths but avoiding those with strong UV reflectance (White et al. 1994; Cutler et al. 1995). *Manduca sexta* and other hawkmoths also show strong olfactory responses (in electroantennogram assays) to a broad array of single compounds and synthetic blends across a range of relevant concentrations (Raguso et al. 1996; Shields & Hildebrand 2001). Laboratory-reared *M. sexta* track floral odour plumes to their sources in the absence of visual cues (Brantjes 1978; Raguso & Willis, in press), and increase the duration of 'searching flights' four-fold (from 2.3 to 8.5 min) in the presence of fragrance (Brantjes 1973). *Manduca sexta* have been trapped in field settings with lures of floral odours (Morgan & Lyon 1928) and learn to associate floral odours with a nectar reward as

quickly and reversibly as do honeybees (Daly & Smith 2000; Daly et al. 2001).

We assessed the relative importance of vision and olfaction in close-range (<5 m) floral attraction and feeding by naïve, laboratory-reared male *M. sexta* by challenging them to forage from arrays of paper flower models and natural flowers. We generated the following mutually exclusive hypotheses to guide our evaluation of the moths' behaviour.

Hypothesis 1

Manduca sexta are attracted over short distances (<5 m) and stimulated to feed by a single sensory cue.

(a) Attraction and feeding are visually guided; odour is superfluous.
(b) Attraction and feeding are olfactorily guided; visual cues are superfluous.

Hypothesis 2

Manduca sexta are attracted over short distances (<5 m) and stimulated to feed on night-blooming flowers by multiple sensory cues, detected sequentially or synchronously.

(a) Attraction is visually guided, but odour functions as a nectar guide or feeding cue (in the presence of visual cues).
(b) Attraction is olfactorily guided, but visual cues function as nectar guides or feeding stimuli (in the presence of odour).

Each hypothesis has precedent in the nectar-feeding behaviour of other Lepidoptera (Weiss 2001; Raguso & Willis, in press) and diverse insect orders (Dobson 1994; Raguso 2001). The predictions listed in Table 1, combined with experimental data, should allow us to distinguish between them. For instance, if each flower must be scented in order to elicit feeding, we could reject hypotheses 1a and 2b and evaluate the remaining hypotheses based on the importance of visual cues. By determining the innate responses of naïve *M. sexta* we hoped to establish a foundation for further studies of both wild and laboratory-reared moths.

METHODS

Study Organisms

We reared *M. sexta* larvae at 25 °C and 50–60% RH under a long day photoperiod (17:7 h light:dark cycle), as
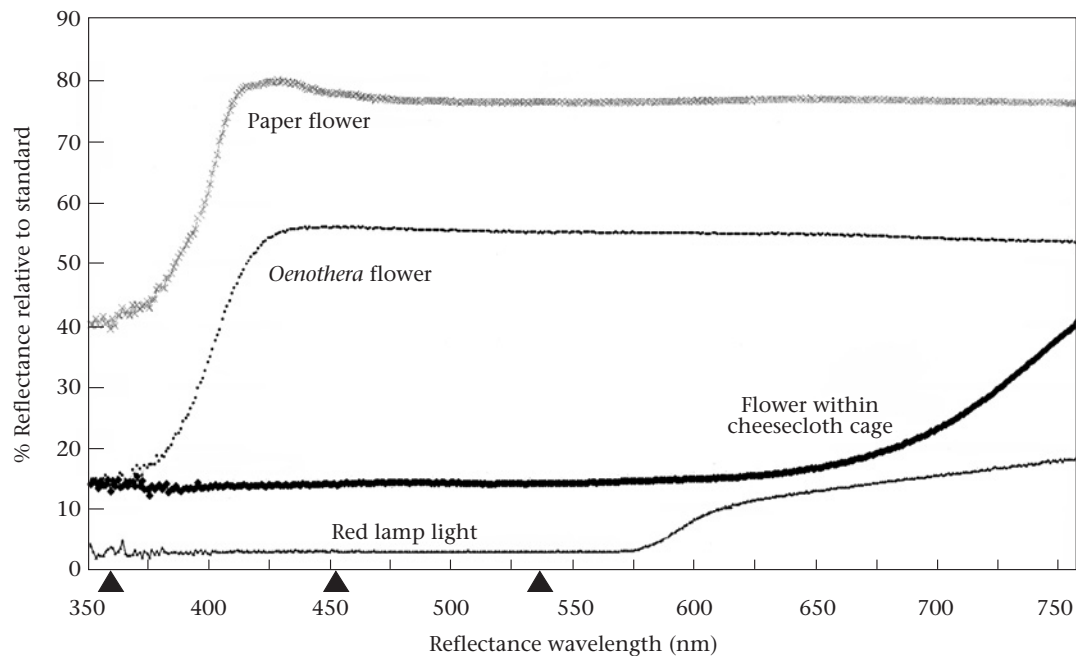
Figure 1. Spectral reflectance curves for visual stimuli used in *M. sexta* foraging experiments. Data are given as a percentage of the reflectance of Duraflect™ pigment (a white optical standard). Data were collected from long UV (350 nm) to IR (800 nm) using a tungsten lamp spectrophotometer and an integration sphere for quantitative comparisons. Arrows at 360, 450 and 560 nm indicate the absorption maxima of *M. sexta*'s three known visual pigments (see text). Note that the cheesecloth effectively masks floral reflectance, paper flowers are more reflective than the *Oenothera* flowers (including UV wavelengths), and the red lamp provides only a small amount of light in wavelengths below 600, including UV.

part of an experimental colony originally established in Oxford, North Carolina, U.S.A. in 1965 (Yamamoto 1969) and maintained since 1987 at the University of Arizona, U.S.A. We fed larvae an artificial diet described by Bell & Joachim (1976). Male pupae were placed in cubic screen cages (35 cm each side) under an LD 14:10 h cycle (temperature during the light phase was 25 °C, and during the dark phase was 20 °C), and adults were held for 2 days without food or exposure to olfactory or visual cues. All assays were performed using male *M. sexta* within the first 90 min of the dark phase, 3 days after eclosion.

Flowers of the evening primrose, *Oenothera neomexicana* (Onagraceae), provided natural odours for our experiments and inspired our white paper models. *Oenothera neomexicana* flowers epitomize the hawkmoth-pollinated mating system common to its genus (Gregory 1964), and potted, flowering plants were available throughout our study. Plants were grown from seed collected on the Swift Trail-Mt Graham Road (Arizona Route 366) at 2500-m elevation in the Pinaleño Mountains, Graham County, Arizona (accession RAR96-4). Vouchers were deposited in the University of Arizona Herbarium. The white flowers of *O. neomexicana* are highly reflective across human-visible wavelengths, but lack intense UV reflectance (Fig. 1). Chemical analysis of the headspace of open flowers identified a complex blend of 26 volatile compounds, including nitrogenous oximes, oxygenated terpenoids, aromatic alcohols and aliphatic esters; all are typical of hawkmoth-pollinated plants worldwide (Kaiser 1993; Knudsen & Tollsten 1993).

Experimental Arena

Experiments were performed at the United States Department of Agriculture (USDA) Carl Hayden Bee Experimentation Laboratory in Tucson, Arizona. Two greenhouses (each 3.75 m long, 2.75 m wide and 2.44 m tall) were used as experimental chambers and were light-proofed with dark vinyl. Each greenhouse was lit by a 25-W red lamp (Philips 'Colortone', wavelengths >600 nm; Fig. 1), equipped with a small fan (120V AC) to provide anticlockwise circulation of plant odours, and splashed with 2 litres of water to increase relative humidity (see Brantjes 1973). We obtained daily maximum and minimum temperatures recorded by the US National Weather Service in Tucson via the internet (http://nimbo. wrh.noaa.gov/Tucson/climate) for use in statistical analyses.

Paper Flower Array with and without Odour

We compared moths' responses to three-by-three arrays of paper flowers with versus without *O. neomexicana* plant odours. Flowers (6-cm diameter) approximated the size, shape and spectral properties of natural *O. neomexicana* flowers (Fig. 1), with 1 ml plastic microcentrifuge tube 'nectaries' filled with 25% sucrose solution. We constructed two arrays (50 × 50 cm) of nine flowers (lateral interfloral distance=25 cm, diagonal distance=33 cm) within black aluminium frames. Arrays were suspended over rectangular cloth cages (35 cm tall, 25 cm deep, 25 cm wide) constructed of brass wire. In the control

greenhouse, the cloth cage was empty, while in the experimental greenhouse it concealed a flowering *O. neomexicana* plant ($\bar{X} \pm$ SE=3.4 ± 0.3 flowers per night). The brown-green dyed cotton cheesecloth (Rit, Inc., Indianapolis, Indiana, U.S.A.) cage visually masked the flowers within (Fig. 1) but permitted odours to waft through the array. The same cage was used to conceal the flowering plant, regardless of greenhouse, to avoid odour contamination. An observer's chair occupied one corner of the greenhouse, 2 m from the array; otherwise both greenhouses were empty. By physically decoupling floral scent and visual cues, we could test the predictions that each of these signals are required for moth feeding. Specifically, if moths feed from model paper flowers in the scentless greenhouse, then scent is not required to initiate feeding. Similarly, if moths probe through the cheesecloth cage at the hidden floral scent source, then visual cues are not required for feeding.

At dusk, we loaded individual moths into cylindrical screen cages (15 cm height, 15 cm diameter) and placed them in an empty greenhouse to acclimatize them to ambient conditions prior to exposure to experimental stimuli. We assigned moths to treatment and greenhouse using a randomized block design, and performed experiments from 19 October to 2 November 1997 until 31 moths had been flown for each treatment. We introduced moths to their assigned greenhouse individually to ensure that subjects could not observe the behaviour of other moths (Slater 1973). Upon release into the greenhouse, the moths wing-fanned for 0.5–3 min (a precursor to flight in large poikilothermic moths; Dorsett 1962; Heinrich & Bartholomew 1971; Willis & Arbas 1991) and then took flight. We allowed each moth to fly for 5 min and continuously scored the number and sequence of behavioural responses using product-Poisson sampling (Colgan & Smith 1978). Moths that landed on the greenhouse walls were re-released 1 min later. In such cases, the 5-min flight time was cumulative, not continuous. After three consecutive landings by an individual (<5% of all cases), observations were terminated and that individual was omitted from statistical analysis.

During flight periods, we scored five behaviours: (1) close pass: flight within 1 m above the array; (2) hover: stationary flight within 1 m above the array; (3) approach to hidden odour source: hover within 10 cm of the cloth cage; (4) floral approach: hover within 10 cm of a flower, clearly orienting to it as a 'target'; (5) probing/feeding: insertion of proboscis into nectar tube.

Thus, 'attraction' consisted of close passes, hovers and approaches to odour. Floral approaches and probing represent subsequent steps beyond 'attraction' in the sequence of nectar feeding. A significant majority of first floral approaches and feeding occurred during the first 2 min (88%; Pearson's chi-square test: χ^2_4=21.5, P<0.001) and 70% of all feeding events were completed in the first 4 min, suggesting that our assays were of sufficient duration for observing the moths' behavioural responses.

We also measured the duration of each hover and the cumulative duration of responses during which moths displayed any of the above behaviours (Martin & Bateson 1993). Mean hover duration per moth was square-root transformed ($y'=\sqrt{(y+0.5)}$) to compensate for Poisson distribution, while total duration of behavioural responses was log transformed ($y'=\log{(y+1)}$) to correct for unequal variances (Zar 1996). We analysed these data as dependent variables, with treatment and greenhouse as fixed factors, the interaction between them as an effect, minimum daily temperature and number of hidden flowers (=odour intensity) as covariates, using fully factorial analysis of covariance (ANCOVA: SYSTAT 5.2.1).

Mixed Array of Natural and Paper Flowers

We designed another floral array, alternating four *O. neomexicana* flowers with five paper flowers in a checkerboard pattern, to test the prediction that scent is required to feed at each flower, as is the case for certain butterflies (Andersson 2001). We modified the arrays to hold glass tubes filled with 35 ml of water to maintain the turgor of excised flowers. We also placed paper flowers into these tubes to control for the presence of water vapour. As before, trays were suspended over rectangular cloth cages that concealed a flowering *O. neomexicana* plant. Thus, fragrance emanated from that source and from individual flowers within the array. We observed the responses of 27 male *M. sexta* to this mixed array, scoring the number and sequence of behaviours during 5-min flight periods, during the same period (24 October–13 November 1997) but on different evenings than the previous experiment. Trials were split evenly between the greenhouses to control for potential differences in lighting, air circulation or other local variables. Again, the 5-min flight time was sufficient, as a significant majority of first approaches and feeding events occurred during the first 2 min (77%; Pearson's chi-square test: χ^2_4=14.2, P<0.005) and all feeding was completed before the end of the fourth minute.

We combined data from both experiments and compared differences in the proportion of moths showing behavioural responses to arrays of scentless paper flowers, paper flowers plus odour, and paper flowers plus natural flowers. Since hovers and close passes were observed in response to all three arrays, we compared the proportion of moths from each treatment performing these behaviours using Pearson's chi-square tests (α=0.05, two-tailed) in 2×3 contingency table analyses. In contrast, the remaining three behaviours did not occur in the absence of odour. We tested whether a significantly greater proportion of moths performed these behaviours when the floral array included natural flowers versus scented paper flowers using Fisher's exact test (α=0.05, one tailed).

Floral Preference and Constancy

If scent is required to feed at each flower, moths should over-visit natural flowers in mixed arrays. We scored the number and sequence of feeding events by each moth in mixed arrays, and tested for homogeneity of preference using the binomial statistic (*S*) described by Jones (1997), to determine whether the feeding sequences of different

moths could be combined for analysis (Waser 1986). We tested for assortative visits to flowers by comparing the proportion of total feeding events at natural flowers to the relative frequency of those flowers in the array (0.44) for each moth using single-sample t tests on arcsine-transformed data (Jones 1997). This constitutes a two-tailed test of the null hypothesis that moths fed from natural and paper flowers according to their relative abundance in the array. Finally, we tested for constancy by asking whether feeding transitions made between natural and paper flowers departed significantly from random expectations. The expected transition frequencies were calculated from the observed proportion of feeding events occurring at natural flowers (Jones 1997). We compared the observed number of 'like' (natural to natural, paper to paper) and 'unlike' transitions to expected values using the chi-square test of independence (Waser 1986; Jones 1997).

Behavioural Sequence Analysis and Ethogram Construction

Nectar feeding by hawkmoths involves a clear sequence of behaviours, from distant to close-range orientation, proboscis extension and feeding (Brantjes & Bos 1980). By presenting moths with floral arrays in which visual and odour cues were decoupled using scented and unscented flower types, we hoped to collect data that would allow us to reject one or more of the hypotheses outlined in Table 1. Behavioural sequence analysis is a powerful ethological tool, especially when combinations of stimuli elicit novel behaviours (Haynes & Birch 1984; Charlton & Cardé 1990). We made continuous recordings of all transitions between the five behaviours observed during each moth's flight period. We constructed 5×5 contingency tables representing transition matrices for each floral array. Table cells represent the conditional probability of a transition between row and column behaviours (Fagan & Young 1978), and were defined as the proportion of moths per treatment showing a specific transition at least once during their flight period (Slater 1973). A cell value of 0.26 indicates that 8 of 31 moths made the row-to-column transition specific to that cell. This approach is preferable to traditional methods for two reasons. First, each cell value is expressed relative to the population of experimental moths, avoiding pseudoreplication. Second, since we did not terminate our trials after the first feeding event, cell values are less likely to violate the assumption of stationarity (stability over time) than mean transitional frequencies calculated from sequences of varying duration (Fagan & Young 1978; Douglas & Tweed 1979).

Some table cells contained 'structural zeros' (transitions that were impossible or biologically meaningless; Slater 1973; Fagan & Mankovich 1980), including the self-transition diagonal and transitions to feeding from close passes, hovers or approaches to hidden odour, since feeding must follow a floral approach. Although maximum likelihood methods are recommended for calculating expected cell values when contingency tables contain structural zeros (Goodman 1968; Bishop 1975; Colgan & Smith 1978; Everitt 1992), we had no explicit Markovian model to test, given the absence of prior studies. Instead, for each table we tested the null hypothesis that row values were independent of column values (random behaviour; Cane 1978), using the ACTUS 2 simulation program (Estabrook & Estabrook 1989). We rounded up proportions to whole numbers and generated expected cell values as described by Estabrook & Estabrook (1989). Cell values were bootstrapped 10 000 iterations, and P values were calculated from the proportion of all simulated tables whose chi-squared values exceeded that of the observed table. When $P<0.05$, ACTUS 2 identified the observed cell values that were significantly higher or lower than expected values (Fagan & Mankovich 1980). To compensate for multiple comparisons (22 cells per table), we adjusted the α value to 0.002 for all post hoc cell-specific evaluations. When table values departed significantly from null expectations, we constructed kinematic diagrams in which the size of the arrow connecting each behaviour represented the conditional probability of that transition (Martin & Bateson 1993; Howard et al. 1998).

RESULTS

Effects of Plant Odours on Attraction to Paper Flowers

Manduca sexta moths were visually attracted to the array of scentless paper flowers, as most individuals (>60%) repeatedly hovered and passed within a metre of the flowers (Table 2). When moths did not respond to these cues, they did not hover over empty space, but flew to the ceiling and attempted to escape, as described by Ramaswamy (1988). The addition of floral odour to the array elicited three novel behaviours: approaches to the hidden odour source, and approaches to and feeding from paper flowers (Table 2). None of the 31 approaches to the hidden odour source were performed with an extended proboscis: moths did not attempt to feed without a visual target. Mean hovering duration was significantly longer when floral arrays were scented, but was not associated with other environmental variables (ANCOVA; Table 3). Also, mean hovering duration was significantly associated with a treatment-by-greenhouse interaction. Despite our efforts to standardize conditions, some unspecified difference between the two greenhouses affected the way that moths responded to odour. Mean duration of behavioural responses was significantly greater when arrays were scented (ANCOVA: $P<0.05$), but did not covary with greenhouse, temperature or number of hidden flowers (Table 3).

Effects of Including Natural Flowers in Array

The addition of natural *O. neomexicana* flowers to the paper flower array resulted in a six-fold increase in the number of feeding events and a significant, three-fold increase in the proportion of moths that fed (Table 2).

Table 2. Summary of *Manduca sexta* behavioural data, all experiments

Behaviour	Treatment	N	Total number of responses	Number of moths that responded (%)	Number of responses/moth ($\bar{X} \pm SE$)
Close pass	Paper flowers	31	106	26 (83.9)	4.1±0.6
	Paper+odour	31	219	30 (96.8)	7.3±0.9
	Mixed array	27	152	23 (85.2)	6.6±0.9
Pearson's chi-square test: χ^2_2=3.08, P=0.22*					
Hover	Paper flowers	31	39	23 (74.2)	1.7±0.1
	Paper+odour	31	86	28 (90.3)	3.1±0.3
	Mixed array	27	78	26 (96.3)	3.0±0.4
Pearson's chi-square test: χ^2_2=8.35, P=0.01*					
Approach odour source	Paper flowers	31	0	0 (0)	0
	Paper+odour	31	31	11 (35.5)	2.8±0.6
	Mixed array	27	13	6 (22.2)	2.2±0.6
Fisher's exact test: P=0.21†					
Approach flower	Paper flowers	31	2	1 (3.2)	2.0
	Paper+odour	31	49	17 (54.8)	2.9±0.5
	Mixed array	27	122	19 (70.4)	6.4±1.3
Fisher's exact test: P=0.17†					
Feed	Paper flowers	31	0	0 (0)	0
	Paper + odour	31	10	5 (16.1)	2.0±0.8
	Mixed array	27	61	15 (55.6)	4.1±0.8
Fisher's exact test: P=0.002†					

*Two-tailed test of the null hypothesis of no difference between proportions of moths showing behavioural responses. Pearson's χ^2 compares responses to all three array treatments.

†One-tailed test of the null hypothesis that adding natural flowers to a scented array would not increase the proportion of moths showing a behavioural response. Fisher's exact test compares responses to paper+odour and mixed array treatments only.

Table 3. Data analysis for paper flower arrays: fully factorial ANCOVA

Dependent variable	Factors and effects	SS	df	MS	F	P	Adjusted least squares* mean (±SE) duration (s)
Mean hover duration (square-root transform)	Odour treatment	5.50	1	5.50	16.72	<0.001	Odourless† 2.5±0.4
	Greenhouse	0.28	1	0.28	0.86	0.36	Plus odour 4.1±0.3
	Treatment*greenhouse	3.15	1	3.15	9.57	0.003	
	Minimum temperature	1.08	1	1.08	3.26	0.08	
	Number of flowers	0.002	1	0.002	0.005	0.94	
	Error	18.43	56	0.33			
Duration of behavioural responses (log transform)	Odour treatment	0.95	1	0.95	6.81	0.012	Odourless 2.2±0.2
	Greenhouse	0.12	1	0.12	0.82	0.37	Plus odour 3.0±0.2
	Treatment*greenhouse	0.003	1	0.003	0.02	0.89	
	Minimum temperature	0.31	1	0.31	2.25	0.14	
	Number of flowers	0.00	1	0.00	0.00	0.98	
	Error		7.84	56	0.14		

*Dependent variable means were adjusted to remove the effects of covariates.

†Values from different treatments are presented as absolute (not relative) measures.

The proportions of moths performing other behaviours did not differ significantly from those observed in response to scented and unscented paper flower arrays (Table 2). Half of all flowers approached were fed upon in mixed arrays, while only 17% of all flowers approached in scented paper flower arrays were fed upon (Fisher's exact test: one-tailed P<0.001, N=181 events). While moths fed more avidly from mixed arrays ($\bar{X} \pm SE$=4.1 ± 0.8 flowers, N=15 bouts) than from scented paper flower arrays ($\bar{X} \pm SE$=2.0 ± 0.8 flowers, N=5 bouts), these differences were not statistically significant (Mann–Whitney U test: U=21.0, χ^2_1=2.18, P=0.14). Similarly, disparities in the mean number of feeding events plus floral approaches (mixed arrays: $\bar{X} \pm SE$=11.8 ± 2.2; scented paper flower arrays: $\bar{X} \pm SE$=7.0 ± 1.7) were not significant (U=22.0, χ^2_1=1.85, P=0.17).
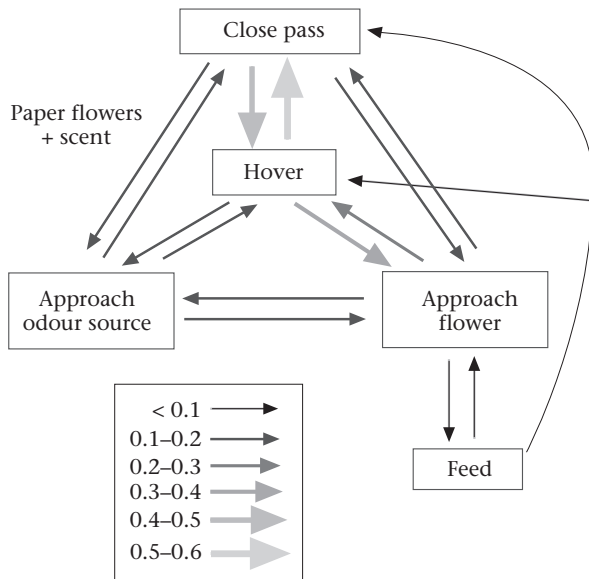
Figure 2. Kinematic diagram (ethogram) of *M. sexta*'s behavioural responses to a scented paper flower array. Arrows represent transition frequencies between five defined behaviours. Increasing arrow width and 'shading' are proportional to magnitude of transition frequency. Note that the dominant transitions are between hovers and close passes.
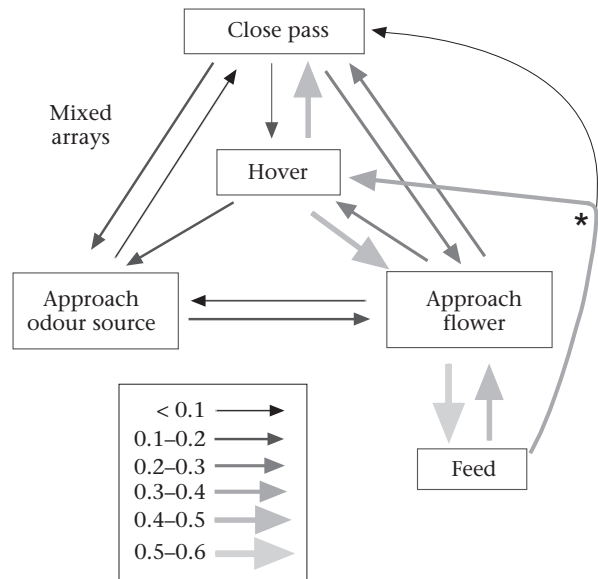
Figure 3. Kinematic diagram (ethogram) of *M. sexta*'s behavioural responses to a mixed array of paper and natural flowers. Note the increased transitions to feeding (cf. Fig. 2) and the flow of behaviour from hovers to floral approaches and feeding, with a return loop (*) representing the continuity of extended floral foraging.

Lack of Discrimination between Natural and Paper Flowers

Moths showed homogeneity of preference while foraging from mixed arrays (feeding only: $S_{10}=7.20$, NS; feeding plus floral approaches: $S_{11}=8.96$, NS), enabling us to combine data from all individuals for further analysis. Of the 15 moths that fed, 12 commenced at a paper flower (Pearson's chi-square test: $\chi^2_1=4.29$, $P<0.05$), a significant departure from null expectations. Once feeding began, the moths showed no preferences for natural or paper flowers, either in feeding (Student's t test: $t_{10}=0.57$, $P=0.58$) or feeding plus approaches ($t_{11}=0.23$, $P=0.82$). Furthermore, differences in the proportion of paper flowers (0.44) versus natural flowers (0.60) that were approached and eventually fed upon did not differ significantly (Fisher's exact test: two-tailed $P=0.13$, $N=122$ events). Finally, we did not observe constancy during feeding bouts, as the number of transitions within and between natural and paper flowers did not depart significantly from null expectations (Pearson's chi-square test: $\chi^2_1=0.184$, $P=0.90$).

Behavioural Sequences and Ethograms

Contingency table analysis revealed no significant behavioural sequence in response to scentless paper flower arrays (Pearson's chi-square test: $\chi^2_2=3.0$, $P=0.14$). With one exception, hovers and close passes never progressed to floral approaches or feeding. When paper flower arrays were scented, behavioural sequences became significantly structured ($\chi^2_{16}=49.92$, $P=0.0001$), including low levels of feeding (Fig. 2). Transitions from floral approaches to feeding were significantly more fre-

quent than expected by chance ($P<0.0001$). In mixed arrays, the behavioural sequences were expanded into iterative feeding bouts ($\chi^2_{16}=105.16$, $P<0.0001$), as hovering more frequently led to floral approaches and feeding (Fig. 3). Transitions from feeding to behaviours conducive to continued feeding (hovers, floral approaches) were significantly more frequent than expected ($P<0.001$), while transitions from feeding to behavioural 'dead ends', such as close passes or approaches to the odour source, were significantly less frequent than expected ($P<0.0001$; Fig. 3).

DISCUSSION

Single-modality Signals are Necessary but Insufficient Feeding Stimuli

Surprisingly, the scentless floral array elicited hovers and close passes by *M. sexta.* These behaviours were not observed when paper flowers were grouped in bouquets (data not shown), suggesting that visual attraction is density dependent, a nearly universal pollinator-attraction strategy (Rathcke 1983; Feinsinger & Tiebout 1991; Smithson & MacNair 1997). Male *M. sexta* also were attracted by odour, as moths hovered beneath the plane of the array and approached the hidden odour source. Such behaviour is consistent with flight tunnel experiments in which naïve *M. sexta* track floral odour plumes upwind to their sources but do not probe them in the absence of visual targets (Raguso & Willis, in press). Furthermore, odour was required for feeding but not as a point source, as *M. sexta* foraged indiscriminately from natural and (unscented) paper flowers within mixed arrays.

These observations allow us to reject both single-modality hypotheses in Table 1; neither odour nor visual cues alone are sufficient to elicit feeding by male *M. sexta.* Furthermore, the dual modality hypothesis of visual attraction and odour-released feeding can be rejected because individual flowers need not be scented if odour is generally present. The remaining hypothesis (odour attraction and visually guided feeding), inspired by Baerends (1950), Tinbergen (1958) and Brantjes (1978) is superficially consistent with our results (Table 1) but is inaccurate because its implied mechanism is sequential. Odour or visual cues alone were redundantly attractive to naïve *M. sexta* males within 5 m of a flower, but simultaneous perception of these stimuli was essential for feeding. Once feeding was initiated, proboscis placement appeared to be visually guided (see Knoll 1925). The significant over-visiting of paper flowers during first visits of feeding bouts was the only example of preference in our experiments. In the presence of fragrance, *M. sexta* may initiate feeding at the most visually contrasting flowers, as does the hummingbird hawkmoth (*Macroglossum stellatarum*: Knoll 1922; Kelber 1997) and many bee species (Lunau & Maier 1995). Indeed, *M. sexta* and other hawkmoths routinely probe at light-emitting diodes, white clothing or rubbish when fragrance is present (Clements & Long 1923; Kugler 1971; Raguso & Willis, in press). However, the decision of whether or not to feed from an individual flower (not directly addressed here) may be based upon sensory information acquired via the proboscis during physical contact with that flower.

Innate Feeding Responses are Synergized by Multimodal Floral Cues

Naïve male *M. sexta* were attracted to flowers by either visual or olfactory stimuli, but approach, proboscis extension and feeding required synchronous detection of both sensory inputs. Since the moths had never seen, smelled nor fed from flowers until their trials began, their initial responses to floral stimuli must have been innate. Ordered behavioural sequences were not observed until visual and olfactory signals were combined, which significantly increased the duration of behaviours associated with floral attraction. When natural flowers were added to arrays, the behavioural flow more closely approximated that of wild moths while feeding (Janzen 1983). Additional experiments will be needed to determine which property of natural flowers (nectar chemistry, increased fragrance, water vapour and CO_2, or reduced UV reflectance) was most responsible for this behavioural shift.

Single-modality signals might elicit feeding under extreme cases of sensory impairment (antennectomy: Clements & Long 1923; visual pigment deficiency: Bennett & White 1989), crowding, starvation (K. A. Snook, personal communication), or associative learning (K. C. Daly, W. L. Mechaber & B. H. Smith, unpublished data), but we have not observed this in our experiments. In previous studies, laboratory-reared *M. sexta* fed from artificial flowers in the absence of floral scent (Yamamoto

1968; Bell & Joachim 1976; White et al. 1994; Cutler et al. 1995), but in each case the flight cage contained a tobacco plant for female oviposition. Some volatiles produced by tobacco vegetation (Sasaki et al. 1984a, b; Andersen et al. 1986) are prevalent in floral scents (Knudsen et al. 1993), albeit at different concentrations and relative abundance. In our field studies, the vegetative odour of *Datura wrightii*, another larval host, was sufficient to elicit feeding by wild and (we must assume) experienced *M. sexta* at paper flowers, but at a lower level relative to moths presented with paper flowers and *Datura* floral scent (Raguso & Willis, in press). Thus, visual targets plus vegetative odours (at least those of larval host plants) are sufficient to elicit feeding behaviour by *M. sexta.* These observations, combined with the lack of evidence for strict convergence of fragrance chemistry among night-blooming plants pollinated by hawkmoths (Kaiser 1993; Knudsen & Tollsten 1993), suggest that olfactory feeding requirements for *M. sexta* may be highly plastic in terms of odour chemistry. Such flexibility is well suited to this multivoltine insect species, which ranges across two continents and collectively uses hundreds to thousands of flower species as nectar sources (Fleming 1970; Schreiber 1978; Pescador 1994).

Multimodal Floral Signals and Plant Pollinator Communication

Visual–olfactory synergism of feeding behaviour is common among flower-visiting Lepidoptera (Scherer & Kolb 1987; Naumann et al. 1991; Honda et al. 1998) and pollinators from other insect orders (Gottsberger & Silberbauer-Gottsberger 1991; Lunau 1992; Roy & Raguso 1997), but its importance can be subtle and unexpected. For example, bumblebees readily visit scentless artificial flowers in laboratory assays (Keasar et al. 1997; Chittka et al. 2001), and the voluminous literature on bumblebee foraging (e.g. Heinrich 1976; Plowright & Laverty 1984) rarely mentions floral scent. Nevertheless, experiments with artificial flowers demonstrate that odour enhances colour discrimination, memory formation and retrieval by *Bombus terrestris* (Kunze & Gumbert 2001). Despite historical single-modality biases among visual and olfactory physiologists, there is a growing appreciation that most pollinators perceive flowers as multimodal sensory entities (e.g. Gegear & Laverty 2001). However, cross-modality neural integration remains poorly understood in any model system (Gerber & Smith 1998; Liu et al. 1999), and animal pollinators are too behaviourally versatile and phylogenetically diverse to bear generalization. Among hawkmoths, the diurnal *M. stellatarum* requires only visual cues to feed (Kelber 1996, 1997; Kelber & Pfaff 1997), while *Amphion floridensis* is lured by fermenting fruit odours (O'Brien 1999) and *Hyles lineata*'s use of sensory cues may vary with light intensity. The innate attraction of nocturnal *M. sexta* to high-contrast objects and plant odours, combined with its feeding response when such signals are paired, yields an insect that can locate, investigate and feed from any open flower in its vicinity in the dead of night. The additional capacity to associate innate visual and olfactory responses with a

specific nectar reward (Daly & Smith 2000; Daly et al. 2001) provides *M. sexta* with a foraging strategy that can adapt readily to changes in nectar availability in an unpredictable floral landscape.

Acknowledgments

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