

Sex-dependent variation in the floral preferences of the hawkmoth *Manduca sexta*

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ARTICLE INFO

Article history:

Received 24 December 2009

Initial acceptance 9 February 2010

Final acceptance 16 April 2010

Available online 8 June 2010

MS. number: A09-00808R

Keywords:

Agave palmeri

Datura wrightii

hawkmoth foraging behaviour

Manduca sexta

pollen load analysis

pollination

sex-specific behaviour

Studies of plant–pollinator interactions have often documented species differences in preferences for floral advertisements and rewards. However, the contribution of intraspecific variation in behaviours, especially between sexes, remains less understood. We explored resource preference and resource use by male and female *Manduca sexta* hawkmoths, relative to two important nectar resources in southern Arizona, U.S.A. *Manduca sexta* is the major pollinator of one of these species (*Datura wrightii*, Solanaceae). Because females must also seek out *D. wrightii* as an oviposition resource, females were predicted to feed upon it more than would males, which should be free to choose the best nectar resource. Using naïve laboratory-reared moths in flight arena experiments, we found that both sexes preferred *Datura wrightii* over *Agave palmeri* (Agavaceae). Exposure to only one species and an odourless paper control, however, revealed sex-specific differences in foraging behaviour, with females feeding longer from *A. palmeri* and males feeding longer from *D. wrightii*, leading us to reject our hypothesis. Differences in feeding preferences directly translated into differences in energy intake. Females gained significantly more energy than did males by feeding from *A. palmeri*. We also examined whether behavioural preferences of moths in the laboratory translated into foraging behaviour in the field. Pollen load analysis of moths caught in 2004 showed that females carried significantly more *A. palmeri* pollen than did males, whereas males carried more *D. wrightii* pollen than did females. Whereas most studies examine pollination associations at the species level, our results highlight the potential importance of between-sex variation in floral visits.

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Flowers provide combinations of visual, olfactory and gustatory cues to advertise the presence of rewards to pollinators (Proctor et al. 1996; Raguso 2004). Floral visitors can use these cues to discriminate amongst co-flowering species within a community (Chittka et al. 1999; Raguso 2004, 2008), as well as to locate individually rewarding flowers (e.g. Weiss 1991; Howell & Alarcón 2007). In certain pollination systems, floral cues also provide additional information to foragers. For example, floral cues are used by highly specialized female yucca moths (*Tegeticula* and *Parategeticula* spp.) to locate host plants (Pellmyr 2003; Svensson et al. 2005), while plant volatiles can act synergistically with sex pheromones to enhance male attraction to and perception of females (Ochieng et al. 2002; Deng et al. 2004). In these interactions, it can be expected that males and females of the same species will differ in their behaviour on flowers, and thus possibly in their effectiveness as pollinators as well. For example, only one sex may interact

mutualistically with the plant (e.g. Pellmyr 2003; Herre et al. 2008), or the quality and/or quantity of floral visits may differ between sexes (e.g. Herrera 1987; Wilson & Thomson 1991). In other cases, differences in male–female size (e.g. Temeles et al. 2000; Temeles & Kress 2003) or phenology (Minckley et al. 1994) may generate differences in floral visits between sexes. To understand the ecology and evolution of plant–pollinator interactions such as these, males and females of the visitor species must be considered separately.

In the semiarid grassland of southern Arizona, the hawkmoth *Manduca sexta* L. (Lepidoptera: Sphingidae) is associated with *Datura wrightii* Regel (Solanaceae) at both the larval and adult stage, as herbivores and pollinators, respectively (Raguso et al. 2003; Bronstein et al. 2007, 2009). In fact, *M. sexta* adults are the most frequent nocturnal visitors to *D. wrightii* at many locations (Raguso et al. 2003; Alarcón et al. 2008a). Both sexes visit the flowers for nectar, with female visits typically taking place just before oviposition on the same plant (Mechaber et al. 2002; Mira & Bernays 2002). *Datura wrightii* shows the typical phenotype of hawkmoth-pollinated flowers, including nocturnal anthesis, intense and sweet fragrance, reflective coloration, and production of copious amounts of sucrose-dominated nectar at the base of the

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funnel-shaped corolla (Grant 1983; Raguso et al. 2003; Riffell et al. 2008).

Rather than exclusively relying on hawkmoth-attracting flowers, a recent study documents that *M. sexta*, along with most of the rest of the Arizona hawkmoth community, extensively uses *Agave palmeri* Engelm. (Agavaceae) as a nectar source (Alarcón et al. 2008a). *Agave palmeri*'s brush-like flowers, hexose-rich nectar, rotten fruit odour, and low flower reflectance have been interpreted as adaptations to attract bats (Howell 1974; Schaffer & Schaffer 1977; Slauson 2000, 2001; Scott 2004; Riffell et al. 2008), and field studies conducted in Arizona have identified nectar-feeding bats as its primary pollinators (Howell & Roth 1981; Slauson 2000, 2001; Scott 2004). These traits, however, do not exclude visits by hawkmoths (Howell 1974; Schaffer & Schaffer 1977; Slauson 2000, 2001; Scott 2004). *Agave palmeri* may in fact be subsidizing *M. sexta* hawkmoth populations with copious amounts of nectar before the onset of the summer monsoon rains, when *D. wrightii* reaches full bloom (Alarcón et al. 2008a; Riffell et al. 2008; Bronstein et al. 2009).

Evidence that *M. sexta* primarily visits *D. wrightii* and *A. palmeri* is based on analyses of pollen carried on the moths' probosces (Alarcón et al. 2008a; Bronstein et al. 2009). Further experiments with male *M. sexta* revealed that they learned to feed from *A. palmeri* umbels, but were innately biased towards *D. wrightii* flowers (Riffell et al. 2008, 2009). However, behavioural preferences of female moths were not examined. Nor did we examine whether differences in feeding preferences between sexes corresponded to sex-based differences in pollen loads in field-collected individuals.

In the present study, we examine sex-based differences in floral visiting behaviour in *M. sexta*. We asked the following questions. (1) In the laboratory, do naïve male and female moths differ in their preference for *A. palmeri* and *D. wrightii* flowers? (2) Do wild-caught male and female *M. sexta* moths differ in their use of *A. palmeri* and *D. wrightii* flowers, as estimated by the pollen that they carry on their probosces? Females are clearly linked through their life history to *D. wrightii* in the Arizona grasslands because it is one of the few acceptable host plants in this region (another host plant, *Proboscidaea parviflora* (Martyniaceae), is not known to be visited for nectar; Mechaber et al. 2002; Mira & Bernays 2002). *Datura wrightii* is patchily distributed in lowland sites, whereas *A. palmeri* can occur in higher densities on the steep upper slopes of hills and mountains and can occur several kilometres away from the *D. wrightii* patches (Alarcón et al. 2008a; Bronstein et al. 2009). Given the distances involved, and the necessity for females to locate dispersed host plant patches, we hypothesized that females would strongly prefer *D. wrightii* over *A. palmeri* in the laboratory, and would be found with *D. wrightii*-biased pollen loads in the field. Males, however, do not face this constraint. Thus, we expected them to choose nectar based exclusively on its food value in the laboratory and in the field.

METHODS

Moth Rearing and Preparation

To test for the existence of sex-specific differences in foraging preferences, we used naïve *M. sexta* adults from a laboratory colony maintained at the Arizona Research Laboratories, Division of Neurobiology at the University of Arizona. Larvae were reared on artificial diet (modified from Bell & Joachim 1976) supplemented with cholesterol (5 g), wheat germ (144 g; 14 mg of carotenoids), cornmeal (140 g; 2 mg of carotenoids), soy (76 g; 10 mg of carotenoids), linseed oil (9 ml) and sugar (36 g) to enhance adult vision. Larvae were reared under a long-day light:dark (LD) regimen (LD 17:7 h) at 25–26 °C and 40–50% relative humidity (RH). Pupae were segregated by sex and held in a rearing room under reverse-

photoperiod conditions (LD 14:10 h) and with a superimposed temperature cycle of LD 26:24 °C. Three days before adult eclosion, pupae were transferred to fibreglass-screen cages (31 × 31 × 32 cm) under 75–85% RH and ambient light conditions.

Flower Characteristics and Sex-specific Preferences by Naïve Moths to Flowers

To establish whether *M. sexta* adults show sex-specific differences in feeding behaviours at *D. wrightii* and *A. palmeri* flowers, we performed behavioural tests on males and females in the summers of 2007 and 2008. Moths had eclosed 3 days prior to testing and were different individuals from those used by Riffell et al. (2008). Naïve male and female moths were kept separate and at no time prior to experimentation were moths exposed to plant odour. We exposed 15–20 moths of each sex to one of the following three sets of choices: (1) a single *D. wrightii* flower versus an *A. palmeri* umbel; (2) a single *D. wrightii* flower versus a paper flower (no-odour control); or (3) an *A. palmeri* umbel versus a paper flower (no-odour control). Moths ($N = 51$ females, $N = 60$ males) were used only once in a single test and then killed by being placed in a waxed bag overnight in a freezer. Experiment 1 tested whether moths of each sex had a preference (defined here as the first flower chosen) between the two species. Experiments 2 and 3 tested whether moths of each sex would feed on a flower from *D. wrightii* or *A. palmeri* when given no other odour choice. Paper flowers were white paper cones with an opening 8 cm in diameter and a length of 18 cm that tapered to an eppendorf tube containing 50 µl of 20% sucrose solution, which approximates the *D. wrightii* standing crop and nectar sugar content; these served as a neutral visual display (see also Riffell et al. 2008). The *D. wrightii* flowers and *A. palmeri* umbels used in the experiments were collected at the field site (Santa Rita Experimental Range; see below) and placed into vials of water 1–2 h before each experiment. Floral headspace collections of the excised flowers revealed that scent emissions did not differ from those of flowers still attached to the plant (unpublished data). Once transported back to the laboratory, flowers were randomly positioned in the flight arena and spaced 1 m apart.

We used one *A. palmeri* umbel and one *D. wrightii* flower for our behavioural experiments because they are the floral units that moths encounter in the field. Furthermore, in terms of display size ($\bar{X} + SE = 10.7 + 0.4$ cm corolla or umbel diameter; two-tailed independent samples t test: $t_{18} = 1.251$, $P = 0.227$) and emitted scent ($\bar{X} + SE = 86.6 + 8.0$ ng/h; two-tailed independent samples t test: $t_{18} = 0.362$, $P = 0.722$), they are functionally equivalent (see Riffell et al. 2008). A *D. wrightii* flower produces an average of 56 µl of sucrose-rich (22%) nectar in an evening, whereas an *A. palmeri* flower produces a 10-fold greater nightly standing crop (616 µl) of hexose (glucose and fructose)-rich (12%) nectar that can sustain hawkmoth hovering times for much longer durations (Riffell et al. 2008). Although *D. wrightii* plants can produce 50–100 flowers on a given night (Raguso et al. 2003) during June–September, they typically produce far fewer ($\bar{X} + SE = 12.8 + 2.4$ open flowers per plant, $N = 12$ plants, 2006 season). In contrast, *Agave palmeri* flowers are always presented in tightly packed umbels ($\bar{X} + SE = 3.2 + 0.4$ umbels with open flowers, $N = 20$ plants at peak bloom during the 2006 season) with several flowers open per umbel at any one time (12–30 flowers, personal observation).

Each experiment was conducted by releasing a single moth into a flight arena (1.8 × 1.8 × 1.8 m) containing the two choices, spaced 1 m apart, from which they could feed (subsequently termed treatments). Foraging behaviours noted for each individual were the flower species at which the first proboscis extension and active feeding took place, the number of proboscis extensions into the corolla, and the time spent feeding on the flower. In addition,

because plant-emitted cues can affect sex-specific behaviours, we also examined whether the flowers stimulated oviposition behaviours by females and abdomen curling by males. Each trial lasted 10 min or until the moth stopped flying for more than 3 min. The moth was then removed from the flight arena. After an interval of at least 5 min, another moth was released into the flight arena. The *D. wrightii* flowers and *A. palmeri* umbels were replaced after every trial.

Energy Intake by Moths per Foraging Bout

To examine whether differences between male and female foraging behaviours may be attributable to energy intake, we examined the amount of energy gained per foraging bout. A foraging bout is defined here as the time that a hawkmoth actively fed from a given flower during a single visit. Two calculations are necessary to determine the energy provided by the flowers per foraging bout: intake rates while feeding from flowers and the nectar volume of energy from flowers of each species. We had previously determined the energy provided by flowers of each species: *D. wrightii* flowers provide about 209 J/flower from sucrose and hexose (glucose and fructose) sugar concentrations in a 56 µl standing crop, and *A. palmeri* flowers provide about 1318 J/flower from sucrose and hexose sugar concentrations in a 616 µl standing crop (Riffell et al. 2008). Using these values, we next determined the intake rates of the feeding moths using two approaches. After each foraging bout, the amount of nectar remaining in the flower was removed and measured using a 7 cm silica needle (model MF28G; World Precision Instruments, Sarasota, FL, U.S.A.). This value was subtracted from the mean amount of nectar sampled within unvisited flowers ($N = 20$). The second method used paper flower mimics (Raguso & Willis 2005; Riffell et al. 2008) with a tube containing 100 µl of 20% sucrose solution in the centre of the flower. Male and female moths were released singly into a flight arena and were allowed to feed from these flower mimics ($N = 16$). Knowing the exact volume of sugar solution in the paper flowers provided an accurate determination of energy intake, while also allowing a comparison of real and paper flowers. Last, to evaluate whether mating experience influenced moth feeding behaviours, a pilot experiment examined the intake rates between mated and unmated males and females. Results from these pilot experiments demonstrated that intake rates between sexes did not differ significantly (two-tailed independent samples t test: $t_{16} = -0.16$, $P = 0.86$), and that intake rates of the 20% sucrose solution closely matched values from the intake rates of male moths feeding from the *D. wrightii* flowers (3.58 and 3.22 µl/s, respectively for males; two-tailed independent samples t test: $t_{36} = 1.26$, $P = 0.21$). Finally, mating experience did not influence intake rates compared to unmated moths (two-tailed independent samples t test for both sexes: $t_{33} = 1.88$, $P = 0.85$). We therefore used an intake rate of 3.22 µl/s and the time moths spent feeding from flowers to calculate the minimum amount of energy gained per foraging bout.

Manduca sexta Collection and Observation in the Field

The field portion of this study was conducted at the University of Arizona's Santa Rita Experimental Range (SRER, N 31.78°, W 110.82°; 1320 m), approximately 45 km south of Tucson, Arizona. The habitat is characterized by rolling semiarid grasslands with seasonally flowering washes, and is bordered by mesquite and oak woodlands. During the summer months (July–September) this area receives an average of 29.2 cm of precipitation and reaches a mean maximum temperature of 31.7 °C (NOAA Western Regional Climate).

In 2004 and 2005, we captured and sexed adult *M. sexta* hawkmoths attracted to collecting lights. In both years collecting

lights were operated once a week at SRER from mid-June through early September, starting before the onset of summer monsoon rains and ending past the peak of adult hawkmoth abundance. The collecting lights consisted of two 175 W mercury vapour lamps, placed on either side of a reflective white sheet, with one 15 W UV blacklight tube hanging from either side of the sheet. Collecting lights were operated for 2 h starting at sunset, when hawkmoths are most active. As each *M. sexta* hawkmoth landed on the sheet we recorded its sex. We removed the pollen carried on its proboscis (see below), and then measured the proboscis and right forewing before transferring the moth to a holding cage. All individuals were released at the end of that evening's census period.

During July and August of 2006 we directly observed and identified hawkmoth floral visitors to *D. wrightii*. Several large *D. wrightii* plants were observed for a total of 20 h. The observation periods commenced at sunset, a period of particularly high hawkmoth activity (Raguso et al. 2003; Alarcón et al. 2008a), and typically lasted 1–2 h. Hawkmoths were allowed to freely visit flowers on a given plant, but as they left we collected them with an aerial net for identification. Moths were identified to species, sexed, and then placed in a holding cage until the end of the observation period, when they were released.

Pollen Collection and Analysis

We removed the pollen that each hawkmoth carried on its proboscis and fixed it on a slide for later examination. To remove pollen, we slowly unrolled the proboscis using forceps that were cleaned with alcohol between uses, and then ran a small (~2 mm³) cube of glycerin jelly containing basic fuchsin stain (Kearns & Inouye 1993) along the dorsal and ventral lengths of the proboscis. The glycerin cube was then placed immediately on a microscope slide, melted over an alcohol lamp, covered with a cover slip, and labelled.

In the laboratory, the fuchsin-stained pollen slides were analysed at 40–100× magnification. Alarcón et al. (2008a) showed that *D. wrightii* and *A. palmeri* heavily dominated the pollen samples, making up 80–90% of all grains carried over 2 years. Therefore, pollen was classified into three groups, *Datura*, *Agave* and Other, with the Other group consisting of all the rarer pollen morphotypes carried by the moths. We then counted the number of pollen grains in each category, and calculated the relative abundance of each group (number of pollen grains of each group/total number of pollen grains) for each individual. Here we only compare *Datura* and *Agave* pollen-load values (arcsine transformed prior to analysis to meet assumptions of normality, but we report backtransformed values).

RESULTS

Naïve Hawkmoth Behaviour

In the laboratory, naïve male *M. sexta* showed a strong preference for *D. wrightii* flowers (as in Riffell et al. 2008). Female *M. sexta* also strongly preferred *D. wrightii* flowers. When placed in the presence of both an *A. palmeri* and a *D. wrightii* flower, moths of both sexes chose *D. wrightii* first significantly more often (G test for goodness of fit: $G_1 = 9.01$, $P < 0.001$; Fig. 1a). Similarly, when male and female moths were placed in an arena with a *D. wrightii* flower and a paper (no-odour control) flower, they always chose and fed from *D. wrightii* (G test for goodness of fit: $G_1 = 13.44$, $P < 0.001$; Fig. 1b). When given a choice between an *A. palmeri* umbel and a paper (no-odour control) flower, males and females both preferred and fed from *A. palmeri* (G test for goodness of fit: $G_1 = 9.01$, $P < 0.001$; Fig. 1c).

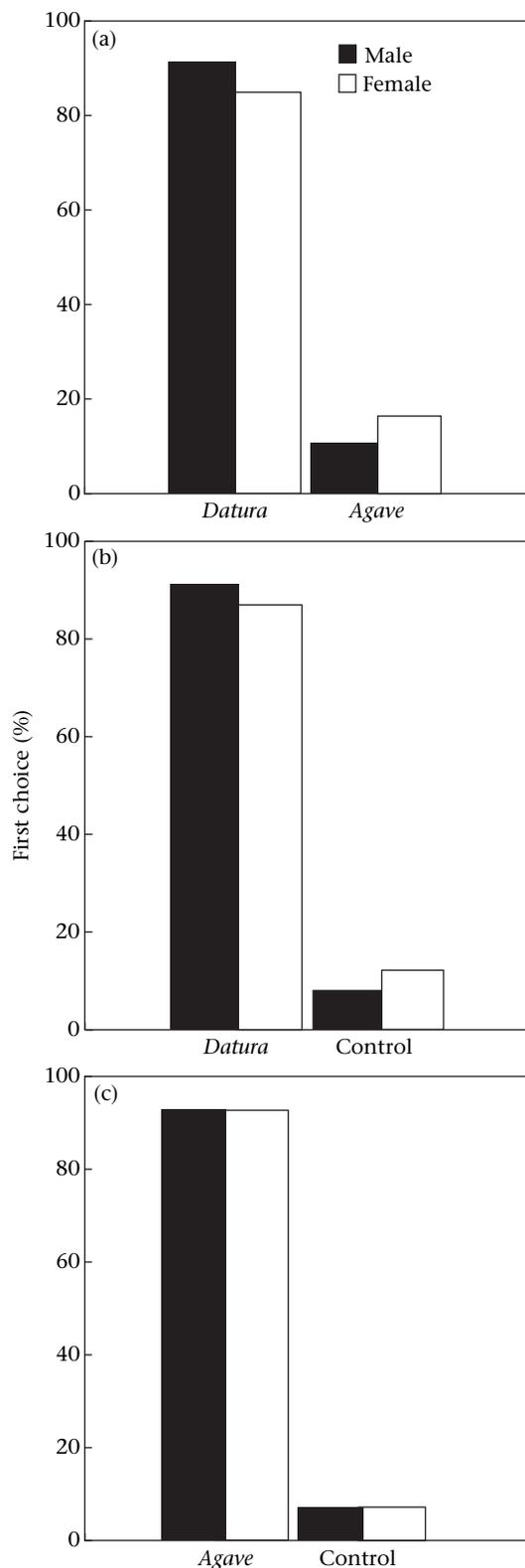


Figure 1. Two-choice experiments examining floral preferences of naïve male and female *M. sexta* moths. Percentages of each sex that chose (a) *Datura wrightii* or *Agave palmeri*, (b) *D. wrightii* or a no-odour control and (c) *A. palmeri* or a no-odour control.

Sex-specific Differences in Behaviours at Flowers

In two-choice experiments with live flowers and paper flower controls in the laboratory, there were significant differences

between sexes in time spent feeding and in the number of proboscis extensions. Overall, moths spent more time feeding from *A. palmeri* flowers than from *D. wrightii* flowers (Table 1). However, there was a difference between the sexes: females spent significantly more time feeding from *A. palmeri* flowers (27.4 s) than males (14.8 s) (post hoc Scheffé test: $P < 0.001$; Fig. 2a, Table 1). By contrast, males fed significantly longer on *D. wrightii* (12.1 s) than females (4.0 s) (Table 1, Fig. 2a), but spent approximately the same time feeding from the two flower species (post hoc Scheffé test: $P = 0.18$; Fig. 2a, Table 1). Males also probed *D. wrightii* flowers more frequently (2.9 probes) than did females (1.1 probes), although the number of times that males and females probed *A. palmeri* did not differ significantly (Table 2, Fig. 2b).

When females came into contact with a *D. wrightii* flower, they spent 48.4% of their time ovipositing upon it and the remaining time (51.5%) feeding from it. In contrast, when in the presence of the *A. palmeri* umbel, females exclusively fed from the flowers; they showed no oviposition behaviour. Males, not surprisingly, showed no sexual behaviours in response to the *D. wrightii* flower or the *A. palmeri* umbel.

Differences between males and females in time spent feeding were directly related to the amount of energy received during those visits, based on nectar intake rates from the flowers (3.22 $\mu\text{l/s}$). On average there was no difference between sexes in total energy ingested from the flowers (two-way ANOVA factor for sex of the moth: $F_{1,67} = 0.11$, $P = 0.73$). However, the amount of energy obtained from the two plant species differed significantly, with *A. palmeri* umbels providing more energy to the moths (sexes combined: 273.6 J) than individual *D. wrightii* flowers (199.6 J) (two-way ANOVA factor for flower: $F_{1,67} = 3.94$, $P = 0.0459$; Fig. 3). Moreover, the interaction between plant species and sex was highly significant (two-way ANOVA factor for sex*flower: $F_{1,67} = 20.23$, $P < 0.0001$). Female moths gained approximately four-fold more energy from *A. palmeri* than from *D. wrightii* flowers, whereas males gained more energy from *D. wrightii* (Fig. 3).

Field Collections and Pollen Analyses

We operated our collection lights at SRER on 12 nights in 2004, resulting in pollen collections from the probosces of 105 *M. sexta* hawkmoths, 28 females and 77 males. In 2005 we operated collection lights for 11 nights and collected pollen from the probosces of 39 moths, 8 females and 31 males. Male and female moths had similar proboscis lengths ($\bar{X} + \text{SE} = 91.3 + 0.7$ mm; two-tailed independent samples *t* test: $t_{141} = 0.407$, $P = 0.685$); however, female moths were larger than males, as estimated by the length of their right forewings ($\bar{X} + \text{SE}$: females: $55.9 + 0.7$ mm; males: $52.0 + 0.3$ mm; $t_{140} = 5.902$, $P < 0.001$). We analysed pollen data for each year separately, since Alarcón et al. (2008a) showed that pollen composition differs between years.

Flowers were abundant, and high numbers of moths were in evidence at the study site in 2004 (Alarcón et al. 2008a). In that year *M. sexta* hawkmoths carried much more *Agave* pollen (~70%) on their probosces than *Datura* (~21%). Females carried significantly more *Agave* pollen (70%) than did males (43%) (two-tailed

Table 1

Two-way ANOVA assessing the effects of moth sex, flower species and their interaction on the time spent feeding from flowers in two-choice experiments with live flowers versus no-odour controls

Factor (df)	Summed square	F	P
Sex (1)	85.67	0.84	0.3671
Flower species (1)	2887.41	27.77	<0.001
Sex × flower species (1)	1809.02	17.40	<0.001
Residuals (67)	103.97		

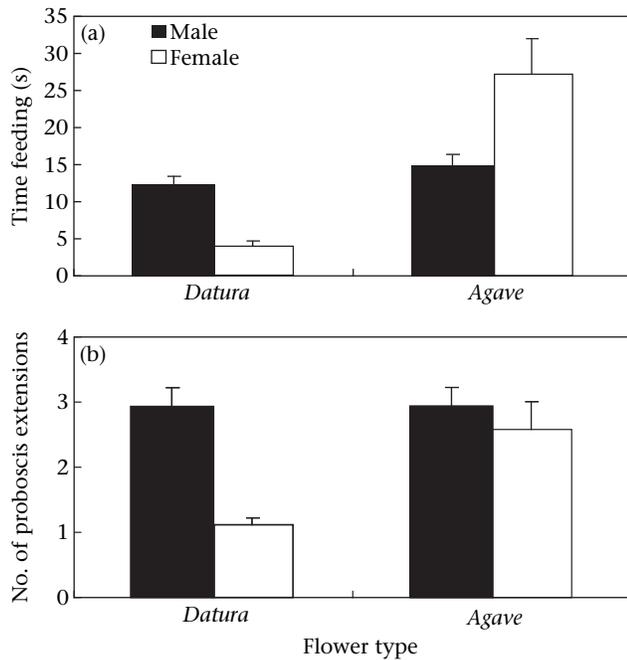


Figure 2. Mean + SE (a) foraging times and (b) proboscis extensions by male and female *M. sexta* moths to live *Datura wrightii* and *Agave palmeri* flowers when given a choice between a live flower of either species and a no-odour (paper) control. Responses to live flowers from each two-choice test are shown.

independent samples *t* test assuming unequal variances: $t_{61.5} = -2.71$, $P = 0.009$; Fig. 4), whereas there was a trend for males to carry more *Datura* pollen (two-tailed independent samples *t* test: $t_{60} = 1.94$, $P = 0.057$) than females (28% vs. 14%; Fig. 4). In 2005, both flowers and moths were extremely scarce, probably because of delayed summer rains (Alarcón et al. 2008a). *Manduca sexta* again carried more *Agave* pollen (~46%) than *Datura* pollen (~31%). However in this case we found no differences between males (45.0%) and females (31.1%) in the amount of *Agave* (two-tailed independent samples *t* test: $t_{37} = 0.546$, $P = 0.546$) and *Datura* (male = 17.9%; female = 45.5%; $t_{37} = -1.248$, $P = 0.220$) pollen carried among the few moths we were able to sample. A power analysis suggested that with this small sample size there was a probability of only 0.09 of detecting a significant difference ($\alpha = 0.05$) in the amount of *Agave* pollen carried by male and female moths in 2005; a sample size of at least 72 moths would have been required to detect a similar difference in *Agave* pollen loads between the sexes (22.8%), as observed in 2004, and significantly more moths would have been required to detect differences in *Datura* ($N \geq 128$) pollen loads, at the levels of significance observed in 2004. Thus, because of the small sample size in 2005, we present data only from 2004 (Fig. 4).

DISCUSSION

Manduca sexta females in southern Arizona have few suitable host plants for oviposition, only one of which, *Datura wrightii*, is

Table 2

Two-way ANOVA assessing the effects of moth sex, flower species and their interaction on the number of proboscis extensions into flower corollas in two-choice experiments with live flowers versus no-odour controls

Factor (df)	Summed square	F	P
Sex (1)	15.09	9.06	0.0036
Flower species (1)	4.64	2.80	0.0990
Sex × flower species (1)	12.06	7.27	0.0089
Residuals (67)	111.17		

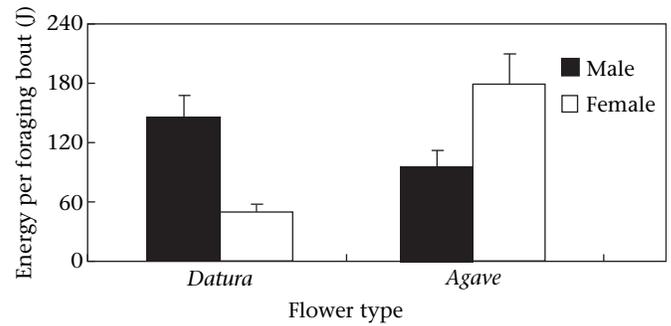


Figure 3. Mean + SE energy provided to male and female *M. sexta* moths per foraging bout for *Datura wrightii* and *Agave palmeri* flowers.

also a useful nectar source. However, a second nectar plant, *Agave palmeri*, is locally abundant in parts of *M. sexta*'s range. We provide evidence elsewhere (Alarcón et al. 2008a) that nectars of both plant species are heavily used by this hawkmoth. Furthermore, we have shown via laboratory experiments that *M. sexta* males prefer to feed on nectar from *D. wrightii* flowers, but that naïve individuals can quickly learn to feed from *A. palmeri* by associating its pungent scent with the copious nectar rewards (Riffell et al. 2008).

Here we tested the hypothesis that preference and utilization of these two nectar plants differed between female and male *M. sexta*. We expected that female moths would prefer *D. wrightii* in behavioural experiments, and that field-captured females would carry *D. wrightii*-biased pollen loads on their probosces. Surprisingly, however, in spite of females' reliance upon *D. wrightii* as an oviposition host plant and, as shown here, a strong innate preference for its nectar in laboratory experiments, females (1) spent more time feeding from *A. palmeri* flowers than from *D. wrightii* in the laboratory, (2) derived more energy from visits to *A. palmeri* than to *D. wrightii* and (3) used *A. palmeri* more heavily in the field than did males. We had expected that male moths would prefer the more rewarding *A. palmeri* in behavioural experiments, and that field-captured males would show evidence that they primarily use *A. palmeri* in spite of an innate preference for *D. wrightii* nectar. Again, contrary to our predictions, we found that (1) male moths spent equivalent time feeding from *D. wrightii* and *A. palmeri* in the laboratory experiments, (2) the difference in energetic sugar content between floral species caused males to derive more energy from their visits to *D. wrightii* and (3) males used *D. wrightii* more in the field than did females based on the pollen loads. Thus, although

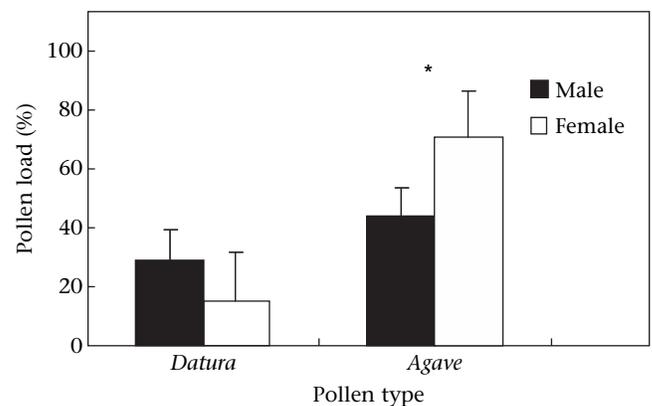


Figure 4. Backtransformed mean (+95% CI) proportion of *Datura* and *Agave* pollen carried on the probosces of male and female *M. sexta* moths in 2004. An asterisk denotes a significant difference in the amount of pollen carried by males and females (*t* test: $P < 0.05$).

both male and female moths strongly preferred *D. wrightii* to *A. palmeri* in controlled two-choice experiments, they used these nectar plants differently when encountering them separately. These behavioural differences translate into higher energy gains for each sex than we had expected.

The innate preference shown by both sexes for *D. wrightii* in the current study demonstrates the importance of this host plant for *M. sexta* in the southwest U.S.A. Females spent equal time ovipositing on and feeding from the *D. wrightii* flower, whereas males only fed from the flower. In contrast, females at *A. palmeri*, which is not a suitable host plant, spent all of their time feeding. Our results suggest that attraction to *D. wrightii*, at least for female moths, may be because the flower is not only a cue of a nectar resource, but also a cue of an appropriate oviposition site. In the vicinity of our study sites, a recent study showed that ovipositing *M. sexta* prefer flowering to nonflowering *D. wrightii* individuals, and that the compound (+)-linalool, identified in the floral scent, mediates this attraction (Reisenman et al., in press). Gravid females, however, might display restraint while feeding at individual *D. wrightii* flowers because they also need to locate and oviposit on dispersed host plants to minimize competition among their offspring, since a single fifth-instar *M. sexta* larvae can consume an entire plant within 48 h (G. Davidowitz, personal observation). Thus, the fact that *M. sexta* females must search for *D. wrightii*, which is patchily distributed in southwestern Arizona, may necessitate their use of *A. palmeri* as an energetic subsidy. Males however, do not have such constraints. Instead, males might benefit from spending more time feeding at *D. wrightii* flowers to increase their probability of encountering females that eclose beneath plants or that arrive to oviposit on them. However, further studies are needed in the field to address this hypothesis.

The between-year differences in pollen load composition (and hence, presumably, in nectar usage) that we identified are probably due to the 10-day delay in the onset of summer rains in 2005 relative to 2004 (Alarcón et al. 2008a). Since hawkmoths typically emerge following the first major rainfall event of the summer, the shift could cause delayed emergence and reduced survival. As indicated by our power analysis, in 2005 we sampled less than 55% of the individuals necessary to detect differences between sexes in pollen load composition. In addition, the floral communities in which hawkmoths foraged in 2005 and 2004 differed greatly. Summer rains in the Sonoran Desert are known to have community-wide effects on flowering abundance and phenology (Bowers & Dimmitt 1994). Furthermore, fewer flowers were produced in 2005 than in 2004 (Alarcón et al. 2008a), which would have forced moths to use less preferred floral resources. In addition, peak flowering of *D. wrightii* occurred about 1 month later in 2005 than in 2004 (Riffell et al. 2008), which probably delayed *M. sexta*'s shift from primarily feeding from *D. wrightii* to *A. palmeri* (Riffell et al. 2008). This could explain why moths carried more *Datura* pollen in 2005 than in 2004. Variation in resource use by pollinators has also been attributed to interannual changes in precipitation patterns in other studies (Alarcón et al. 2008b). However, further work is necessary to document resulting behavioural shifts in resource use and the fitness consequences for insect pollinators.

The sex-based differences we have documented may also be influenced by differences in olfactory capabilities between the sexes. Female *M. sexta* olfactory receptor cells (ORCs) are more sensitive than those of males to plant-derived volatiles (Fraser et al. 2003). In contrast, the majority of male ORCs are sensitive to pheromonal compounds emitted by females (Christensen et al. 1989; Kaissling et al. 1989). These differences in ORC sensitivity suggest that females may be more likely to explore novel flowers for nectar compared to male moths; however, this needs to be verified in the field.

Foraging Strategies and Sex-specific Differences in Metabolic Demands

The metabolic demand of reproduction is also an important factor mediating behaviours of many insects (Wheeler 1996; Papaj 2000), and in combination with the energetic requirements of other adult behaviours, may underlie the observed foraging differences between sexes in *M. sexta*. The energy invested by females into eggs is one example of the costs associated with this process. For example, in the house fly, *Musca domestica*, and ladybird beetle, *Adalia bipunctata*, the energy content for eggs weighing 0.12 and 0.1 mg is estimated to be 0.212 and 0.795 J/egg, respectively (McIntyre & Gooding 2000; Fernandes & Briegel 2004). Although the eggs of these insects are 10-fold lower in mass than *M. sexta* eggs (1.2 mg), they provide a coarse estimate for the energetic content of eggs. If female *M. sexta* eggs have similar energy content, and a female lays 100–300 eggs in an evening (Mechaber et al. 2002), then females may lose 4–239 J during oviposition. The fat body (the energy storage site) of adult *M. sexta* females is nearly double that of males, as is female body size and metabolic demand for flight relative to males (68 and 121 cm³/h oxygen consumption rate for male and female moths, respectively; Bartholomew & Casey 1978). Males, on the other hand, may lose substantially less during reproduction. In the monarch butterfly (*Danaus plexipus*) and bush crickets (Orthoptera: Tettigoniidae), about 10⁻⁶–10⁻⁵ J are invested into sperm and ejaculate (Oberhauser 1992; Wedell 1994). Given that 50–60% of the energy content of eggs comes directly from the adult nectar diet for the hawkmoth *Amphion floridensis* (O'Brien et al. 2000), the higher combined metabolic cost of flight with egg investment may require female moths to use different behavioural strategies for floral resources than males. Further experiments examining the lipid, carbohydrate and protein content of *M. sexta* eggs and sperm, as well as behavioural experiments examining the metabolic costs of flight between sexes are needed.

Sex-specific Differences in Pollinator Quality

Manduca sexta is the most important pollinator of *D. wrightii* in this southern Arizona habitat (Bronstein et al. 2009). Differences in male and female preferences for, and visits to, *D. wrightii* flowers are likely to reflect important sex-based differences in the qualities of each sex as pollinators. Our results suggest that male *M. sexta* may be more effective pollinators than females because: (1) they apparently spend more time feeding from *D. wrightii* relative to other nectar sources, (2) they carry more *D. wrightii* pollen in some years than females, and (3) their visits do not directly lead to costs associated with the deposition of herbivorous offspring. However, it remains to be determined whether male and female *M. sexta* differ in the quantity and quality of *D. wrightii* pollen that they move between flowers within and among plants.

In pollination biology as a whole, traits of floral visitors are almost always considered at the species level. Yet, there is extensive evidence for within-species differences in pollinator quality. Body size can correlate with pollen receipt and deposition, for example (Steiner & Whitehead 1991). With regard to sex-based differences, male and female purple-throated carib hummingbirds, *Eulampis jugularis* (Trochilidae), in the Lesser Antillean Islands prefer to feed upon and pollinate species of *Heliconia* (Heliconiaceae), whose flowers correspond to the length and curvature of each sex's bill (Temeles et al. 2000; Temeles & Kress 2003). Among most solitary bee species, males typically visit flowers to feed on nectar, whereas female bees must additionally collect pollen to provision their offspring (Michener 2000). These differences have the potential to translate into differential quality as pollinators (Herrera 1987; Wilson & Thomson 1991). In other cases, only one sex acts as

a pollinator. For example, only female yucca moths and fig wasps actively collect and deposit pollen, carrying out this activity in the course of oviposition (Pellmyr 2003; Herre et al. 2008). Male euglossine bees (Apidae) collect fragrant compounds from tropical orchids, and in doing so function as their primary pollinators; these flowers are not visited by female euglossine bees (Eltz et al. 1999). Male *Andrena nogroaenea* bees (Andrenidae) pollinate the sexually deceptive orchid *Ophrys sphegodes* through pseudocopulation with flowers that emit volatile compounds similar to the sex pheromones of virgin *A. nogroaenea* females (Schiestl et al. 1999). Beyond any behavioural differences, males and females may have different phenologies, and this alone can affect their quality as pollinators (Minckley et al. 1994). In combination with this body of work, our results suggest that further attention should be given to how selection shapes both plant and animal traits in the context of between-sex variation in floral visiting patterns within species.

Acknowledgments

The work reported here was supported by National Science Foundation grants DEB-0316205 and IOS-0822709, by National Institutes of Health grant DC-02751, by the Postdoctoral Excellence in Research and Teaching (PERT) Fellowship through the National Institutes of Health training grant no. 2 K12 G000708-06, and by a seed grant from University of Arizona's Center for Insect Science. We especially thank Jenny Barker, Greg Barron-Gafford, Erick Chen, Benjamin Collins, Ilan Davidowitz, Sarah Diamond, Jenny Graber, Bryan Helm, Brianna Horvath, Alice Levine, Jack Lin, Kelly Mackay, Connie Meyers, Hillary Miller, Jim Pearson, Virginia Pham, Kristen Potter, Benjamin Pri-Tal, Rebecca Ruppel, Valerie Rychka, Elliot Saperstein, Ratchanee Sananmuang, Francisco Santa Maria, Karla Scott, David Sung, Alex Urquidez and Maria Williams, who provided invaluable assistance in blacklighting. Arturo Acosta, Jennifer Graber, Francisco Santa Maria and Alex Urquidez helped to analyse pollen. We also thank the staff of the Santa Rita Experimental Range for facilitating our use of this site.

References

- Alarcón, R., Bronstein, J. L. & Davidowitz, G. 2008a. Nectar usage in a southern Arizona hawkmoth community. *Ecological Entomology*, **33**, 503–509.
- Alarcón, R., Waser, N. M. & Ollerton, J. 2008b. Year-to-year variation in the topology of a plant–pollinator interaction network. *Oikos*, **117**, 1796–1807.
- Bartholomew, G. A. & Casey, T. M. 1978. Oxygen consumption of moths during rest, pre-flight warm-up, and flight relation to body size and wing morphology. *Journal of Experimental Biology*, **76**, 11–25.
- Bell, R. A. & Joachim, F. G. 1976. Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Annals of the Entomological Society of America*, **69**, 365–373.
- Bowers, J. E. & Dimmitt, M. A. 1994. Flowering phenology of six woody plants in the northern Sonoran Desert. *Bulletin of the Torrey Botanical Club*, **121**, 215–229.
- Bronstein, J. L., Huxman, T. E. & Davidowitz, G. 2007. Plant-mediated effects linking herbivory and pollination. In: *Ecological Communities: Plant Mediation in Indirect Interaction Webs* (Ed. by T. Ohgushi, T. G. Craig & P. W. Price), pp. 79–103. Cambridge: Cambridge University Press.
- Bronstein, J. L., Huxman, T. H., Horvath, B., Farabee, M. & Davidowitz, G. 2009. Reproductive biology of *Datura wrightii*: the benefits of associating with an herbivorous pollinator. *Annals of Botany*, **103**, 1435–1443.
- Chittka, L., Thompson, J. D. & Waser, N. M. 1999. Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften*, **86**, 361–377.
- Christensen, T. A., Hildebrand, J. G., Tumlinson, J. H. & Doolittle, R. E. 1989. Sex pheromone blend of *Manduca sexta*: responses of central olfactory interneurons to antennal stimulation in male moths. *Archives of Insect Biochemistry and Physiology*, **10**, 281–291.
- Deng, J. Y., Wei, H. Y., Huang, Y. P. & Du, J. W. 2004. Enhancement of attraction to sex pheromones of *Spodoptera exigua* by volatile compounds produced by host plants. *Journal of Chemical Ecology*, **30**, 2037–2045.
- Eltz, T., Whitten, W. M., Roubik, D. W. & Linsenmair, K. E. 1999. Fragrance collection, storage, and accumulation by individual male orchid bees. *Journal of Chemical Ecology*, **25**, 157–176.
- Fernandes, L. & Briegel, H. 2004. Reproductive physiology of *Anopheles gambiae* and *Anopheles atroparvus*. *Journal of Vector Ecology*, **30**, 11–26.
- Fraser, A. M., Mechaber, W. L. & Hildebrand, J. G. 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant head-space volatiles. *Journal of Chemical Ecology*, **29**, 1813–1833.
- Grant, V. 1983. The systematic and geographical distribution of hawkmoth flowers in the temperate North American flora. *Botanical Gazette*, **144**, 439–449.
- Herre, E. A., Jandér, K. C. & Machado, C. A. 2008. Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 439–458.
- Herrera, C. M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos*, **50**, 79–90.
- Howell, D. J. 1974. Bats and pollen, physiological aspects of the syndrome of chiropterophily. *Comparative Biochemistry and Physiology*, **48**, 263–276.
- Howell, A. D. & Alarcón, R. 2007. *Osmia* bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour*, **74**, 199–205.
- Howell, D. J. & Roth, B. S. 1981. Sexual reproduction in agaves: the benefits of bats; the cost of semelparous advertising. *Ecology*, **62**, 1–7.
- Kaissling, K.-E., Hildebrand, J. G. & Tumlinson, J. H. 1989. Pheromone receptor cells in the male moth *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, **10**, 273–279.
- Kearns, C. A. & Inouye, D. W. 1993. *Techniques for Pollination Biologists*. Niwot: University Press of Colorado.
- McIntyre, G. S. & Gooding, R. H. 2000. Egg size, contents, and quality: maternal-age and -size effects on house fly eggs. *Canadian Journal of Zoology*, **87**, 1544–1551.
- Mechaber, W. L., Capaldo, C. T. & Hildebrand, J. G. 2002. Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to hostplant volatiles change with age and mating status. *Journal of Insect Science*, **2**, 1–8.
- Michener, C. D. 2000. *The Bees of the World*. Baltimore: Johns Hopkins University Press.
- Minckley, R. L., Wcislo, W. T., Yanega, D. & Buchmann, S. L. 1994. Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. *Ecology*, **75**, 1406–1419.
- Mira, A. & Bernays, E. A. 2002. Trade-offs in host use by *Manduca sexta*: plant characters vs natural enemies. *Oikos*, **97**, 387–397.
- Oberhauser, K. S. 1992. Rate of ejaculate breakdown and intermating intervals in monarch butterflies. *Behavioral Ecology and Sociobiology*, **31**, 367–373.
- O'Brien, D. M., Schrag, D. P. & Martinez del Rio, C. 2000. Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology*, **81**, 2822–2831.
- Ochieng, S. A., Park, K. C. & Baker, T. C. 2002. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. *Journal of Comparative Physiology A*, **188**, 325–333.
- Papaj, D. R. 2000. Ovarian dynamics and host use. *Annual Review of Entomology*, **45**, 423–448.
- Pellmyr, O. 2003. Yuccas, yucca moths, and coevolution: a review. *Annals of the Missouri Botanic Garden*, **90**, 35–55.
- Proctor, M., Yeo, P. & Lack, A. 1996. *The Natural History of Pollination*. London: Harper–Collins.
- Raguso, R. A. 2004. Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology*, **7**, 434–440.
- Raguso, R. A. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Annual Review of Ecology, Evolution and Systematics*, **39**, 549–569.
- Raguso, R. A. & Willis, M. A. 2005. Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths. *Animal Behaviour*, **65**, 407–418.
- Raguso, R. A., Henzel, C., Buchman, S. L. & Nabhan, G. P. 2003. Trumpet flowers of the Sonoran Desert: floral biology of *Peniocereus* cacti and sacred *Datura*. *International Journal of Plant Sciences*, **164**, 877–892.
- Reisenman, C. E., Riffell, J. A., Bernays, E. A. & Hildebrand, J. G. In press. Antagonistic effects of floral scent in an insect–plant interaction. *Proceedings of the Royal Society B*. doi:10.1098/rspb.2010.0163.
- Riffell, J. A., Alarcón, R., Abrell, L. A., Davidowitz, G., Bronstein, J. L. & Hildebrand, J. G. 2008. Behavioral consequences of innate preferences and olfactory learning in hawkmoth–flower interactions. *Proceedings of the National Academy of Sciences, U.S.A.*, **105**, 3404–3409.
- Riffell, J. A., Lei, H., Christensen, T. A. & Hildebrand, J. G. 2009. Characterization and coding of behaviorally significant odor mixtures. *Current Biology*, **19**, 335–340.
- Schaffer, W. M. & Schaffer, M. V. 1977. The reproductive biology of Agavaceae: I. Pollen and nectar production in four Arizona Agaves. *Southwestern Naturalist*, **22**, 157–167.
- Schiestl, F. P., Ayasse, M., Paulus, H. F., Lofstedt, C., Hansson, B. S., Ibarra, F. & Franck, W. 1999. Orchid pollination by sexual swindle. *Nature*, **399**, 421–422.
- Scott, P. E. 2004. Timing of *Agave palmeri* flowering and nectar feeding bat visitation in the Peloncillos and Chiricahua mountains. *Southwestern Naturalist*, **49**, 425–434.
- Slauson, L. A. 2000. Pollination biology of two chiropterophilous Agaves in Arizona. *American Journal of Botany*, **87**, 825–836.
- Slauson, L. A. 2001. Insights on the pollination biology of Agave (Agaveaceae). *Haseltonia*, **8**, 10–23.
- Steiner, K. E. & Whitehead, V. B. 1991. Oil flowers and oil bees: further evidence for pollinator adaptation. *Evolution*, **45**, 1493–1501.
- Svensson, G. P., Hickman, M. O., Jr, Bartram, S., Boland, W., Pellmyr, O. & Raguso, R. A. 2005. Chemistry and geographic variation of floral scent in *Yucca filamentosa* (Agavaceae). *American Journal of Botany*, **92**, 1624–1631.

- Temeles, E. J. & Kress, W. J.** 2003. Adaptation in a plant–hummingbird association. *Science*, **300**, 630–633.
- Temeles, E. J., Pan, I. L., Brennan, J. L. & Horwitt, J. N.** 2000. Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science*, **289**, 441–443.
- Wedell, N.** 1994. Dual function of the bushcricket spermatophore. *Proceedings of the Royal Society B*, **258**, 181–185.
- Weiss, M. R.** 1991. Floral color change as cues for pollinators. *Nature*, **354**, 227–229.
- Wheeler, D.** 1996. The role of nourishment in oogenesis. *Annual Review of Entomology*, **41**, 407–431.
- Wilson, P. & Thomson, J. D.** 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology*, **72**, 1503–1507.