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The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*

Heidy L. Contreras · Joaquin Goyret · Martin von Arx · Clayton T. Pierce ·
Judith L. Bronstein · Robert A. Raguso · Goggy Davidowitz

Abstract The foraging decisions of flower-visiting animals are contingent upon the need of an individual to meet both energetic and osmotic demands. Insects can alter their food preferences to prioritize one need over the other, depending on environmental conditions. In this study, preferences in nectar sugar concentrations (0, 12, 24 %) were tested in the hawkmoth *Manduca sexta*, in response to different levels of ambient humidity (20, 40, 60, and 80 % RH). Moths altered their foraging behavior when placed in low humidity environments by increasing the volume of nectar imbibed and by consuming more dilute nectar. When placed in high humidity environments the total volume imbibed decreased, because moths consumed less from dilute nectars (water and 12 % sucrose). Survivorship was higher with higher humidity. Daily foraging patterns changed with relative humidity (RH): moths maximized their nectar consumption earlier, at lower humidities. Although ambient humidity had an impact on foraging activity, activity levels and nectar preferences, total energy intake was not affected. These results show that foraging decisions made by *M. sexta* kept under different ambient RH levels allow individuals to meet their osmotic demands while maintaining a constant energy input.

Keywords Feeding behavior · Hawkmoths · *Manduca sexta* · Nectar preference · Osmoregulation

Abbreviations

C	Celsius
Cal	Calories
dH$_2$O	Distilled water
g	Gram
h	Hour
kJ	Kilo joule
kcal	Kilo calories
L:D	Light:dark
MV	Mercury vapor
RH	Relative humidity
V	Volume
w/w	Weight/weight
w/v	Weight/volume
SRER	Santa Rita Experimental Range

H. L. Contreras (✉) · M. von Arx · C. T. Pierce ·
G. Davidowitz
Department of Entomology, University of Arizona,
Tucson, USA
e-mail: hcontreras@laverne.edu

G. Davidowitz
e-mail: goggy@email.arizona.edu

H. L. Contreras
Biology Department, University of La Verne, La Verne, USA

J. Goyret · R. A. Raguso
Department of Neurobiology and Behavior, Cornell University,
Ithaca, USA

J. L. Bronstein
Department of Ecology and Evolutionary Biology,
University of Arizona, Tucson, USA

Introduction

Due to limited water sources and an environment that promotes evaporative water loss, one of the greatest challenges faced by terrestrial organisms is maintaining a proper internal water balance (Hadley 1994; Bradley 2009). In terrestrial insects, osmoregulatory adaptations include a waterproofed cuticle and the capacity to produce

hyperosmotic urea (Bradley et al. 2009). Yet, insects may still lose over 80 % of body water through evaporative water loss (Chown 2002). Therefore, some terrestrial insects may also exhibit traits that aid them in acquiring water from the environment. For example, the desert cockroach, *Arenivaga investigata*, can remove water from subsaturated air using bladder-like extensions of the hypopharynx (O'Donnell 1977). Other insect species have the capacity to extract water from air using the rectum (Hadley 1994; Bradley 2009).

Behavioral adaptations for efficient water acquisition often are pronounced in insects found in arid environments. For example, a Namib Desert tenebrionid beetle uses a head-down posture to harvest fog droplets that condense on its body (Hamilton and Seely 1976). Some insects may also alter their foraging behaviors to meet osmotic demands (Watt et al. 1974; Ohguchi and Aoki 1983; Willmer 1986, 1988). The honey bee, *Apis mellifera*, frequently gathers water and diluted nectar under hot or arid conditions (Butler 1940, 1974; Free 1977) or uses regurgitated nectar droplets for evaporative cooling (Cooper et al. 1985). Although individual bees prefer to feed on nectar containing 40 % sucrose, when the colony's need for water is increased individuals' preferences switch to more dilute nectar containing 20 % sucrose (Ohguchi and Aoki 1983). Similarly, the mason bee *Chalicodoma sicula* feeds from flowers containing dilute nectars when ambient conditions are dry, but favors flowers containing higher sugar content under more humid conditions (Willmer 1986). Insect foraging decisions, therefore, appear to be a compromise between an individual's need for sugar (as an energy source) and for water (to maintain proper internal water balance). Osmoregulation is a matter of maintaining a balance of ions as well as water. This study is focused on water and sugar; therefore, we refer to osmotic demands only in the context of water balance.

In the Sonoran Desert, as in other desert environments, dramatic changes in ambient relative humidity (RH) can be observed over both space and time (Watson et al. 1994; Unland et al. 1996; Adams and Comrie 1997). The summer monsoon, which brings in warm moist air from the gulf of Mexico to the southeast, produces about 40–60 % of the Sonoran Desert's annual rainfall (Douglas et al. 1993; Davidowitz 2002), but the temporal frequency and geographic distribution of these summer thunderstorms vary considerably across seasons and among years (Watson et al. 1994; Adams and Comrie 1997; Davidowitz 2002). Changes in RH can affect the energy content provided by nectar as nectar concentrations increase with decreasing ambient humidity (Corbet and Delfosse 1984). The Southwestern United States has been identified as a biodiversity hotspot by Conservation International (Mittermeier et al. 2004) that supports one of the most diverse insect communities in North America (Brusca and Moore 2013), including many species of largely tropical hawkmoths (Lepidoptera: Sphingidae) at the northern limits of their distributions (Hodges 1971). One of these species, *Manduca sexta*, is distributed across the Americas and is common in the Sonoran Desert, where it is subjected to marked changes in ambient humidity throughout the monsoon season, when the moths are most active (Tuttle 2007; Alarcón et al. 2008). Although *M. sexta* utilizes a variety of floral nectar sources throughout its range, in the Sonoran Desert it shows an innate preference for flowers of its host plant *Datura wrightii* (Solanaceae) (Alarcón et al. 2008, 2010; Riffell et al. 2008). These large, night-blooming flowers produce sucrose-rich nectar with ~ 21 to 25 % (w/v) sugar concentrations (Raguso et al. 2003). Flowering *D. wrightii* are patchily distributed at the landscape scale (Alarcón et al. 2008), and their availability changes throughout the monsoon season (Raguso et al. 2003). When *D. wrightii* flower density is low, *M. sexta* and other hawkmoths readily feed from the bat-pollinated flowers of *Agave palmeri* (Agavaceae) (Alarcón et al. 2008, 2010; Riffell et al. 2008). Flowers of *A. palmeri* produce tenfold larger volumes of nectar than flowers of *D. wrightii*, but the nectar is more dilute (12 % w/v) and is dominated by hexose sugars (glucose and fructose) rather than sucrose. Although *M. sexta* moths show an innate preference for flowers of *D. wrightii*, they can learn to feed from flowers of *A. palmeri*, and continue feeding from both species opportunistically throughout their lifetimes (Riffell et al. 2008).

In general, hawkmoth pollinated flowers produce sucrose-dominated nectar (Baker and Baker 1982, 1983; Haber and Frankie 1982). In nature, most hawkmoths feed from flowers producing nectar with sugar concentrations of about 22 % (w/w) (Pyke and Waser 1981; Heyneman 1983), the most common nectar concentration in nocturnal flowers. However, hawkmoths have been reported to feed from flowers containing sugar concentrations ranging from 10 to 44 % (w/w) (Pyke and Waser 1981; Haber and Frankie 1989). In controlled laboratory experiments, the diurnal hawkmoth *Macroglossum stellatarum* prefers to feed from sucrose solutions (Kelber 2003) with optimal concentrations varying between 30 and 40 % (w/v) (Josens and Farina 1997, 2001). Compared with monosaccharide-based nectars, sucrose-rich nectars provide hawkmoths with double the amount of energy per unit volume (Watt et al. 1974; Beuchat et al. 1990; Nicolson 1998). Hawkmoths, which normally feed while hovering, have been shown to increase their metabolism 100-fold from their standard resting metabolic rates during hovering flight (Heinrich 1971; Heinrich and Casey 1973; Bartholomew and Casey 1978). In *M. sexta*, oxygen consumption during flight has been measured between 45 and 50 mL O_2 g/h at

ambient temperatures of 15–30 °C (Heinrich 1971). In other words, a moth weighing 2 g on average expends about 8.3 cal/min (34 J/min) while hovering. Therefore, for these large flying insects, sucrose-rich nectars provide a twofold benefit: higher energy content and lower osmoregulatory costs.

Foraging decisions in *M. sexta*, as in other desert-inhabiting insects, should reflect the moth's need to meet both energy and water demands. Ambient RH has been shown to increase activity levels of small insects (Juillet 1964; Kaspari and Weiser 2000; Zhang et al. 2008; Liu et al. 2011). If this is true for *M. sexta*, the need to meet energetic demands might be prioritized in a humid environment (predicting a preference for concentrated nectars), whereas osmotic demands might take priority in dry environments (predicting a preference for dilute nectars or water supplementation; Raguso et al. 2005). As RH in the Sonoran Desert increases dramatically with the onset of the monsoons, higher activity may lead to greater moth abundances with the onset of the monsoons. We, therefore addressed four questions in this study. (1) Do seasonal changes in RH affect hawkmoth abundances in the wild? (2) Does ambient RH affect flight activity? (3) Does ambient RH affect feeding behavior? (4) Does ambient RH affect preference for sugar concentration?

Materials and methods

Field survey: *M. sexta* abundance as a function of ambient humidity

To determine how hawkmoth abundance changes with ambient humidity in the wild, we identified all hawkmoths that came to a mercury vapor (MV) light trap once a week from June to October 2005 at a grassland study site in the Santa Rita Experimental Range (SRER) 40 km south of Tucson, AZ (for details see Alarcón et al. 2008, 2010; Riffell et al. 2008). Ambient RH at the site was recorded with a temperature/relative humidity data logger (HOBO U23 Pro V2 External Temperature/Humidity Data Logger; Onset, Bourne, MA, USA). We plotted the pattern of changes in RH and hawkmoth abundances across the season. We used simple linear regression to test for statistical significance of overall hawkmoth abundance, *M. sexta* abundance, and hawkmoth diversity (Shannon–Wiener Index) as a function of ambient RH.

Experimental animals for laboratory studies

Manduca sexta used in our laboratory studies were obtained from a colony maintained at the University of Arizona in Tucson, AZ (see Raguso and Willis 2002;

Davidowitz et al. 2004, 2012). Larvae were raised under a 16:8 light:dark photocycle in an environmentally controlled room set at 27 °C and 40–50 % RH. Larvae, separated in individual bins depending on developmental stage, were fed artificial diet (modified from Bell and Joachim 1976) ad libitum until pupation. Pupae were segregated by sex and were held in a rearing room under ambient conditions similar to those experienced as larvae. Three days before adult eclosion, individual pupae were separated and placed in brown, waxed, paper bags until adults emerged. Bags were checked daily, so that newly emerged adults were quickly placed under experimental conditions.

Flight activity experiment

Flight activity of *M. sexta* was assessed at four RH levels (20, 40, 60, 80 % RH). Moths were handled as described above and kept singly in cages, with ad libitum access to three feeders (dH$_2$O, 12 and 24 % sucrose solutions). The flight activity of eight moths (four females and four males) in each RH treatment was recorded with a video camera (HDR-CX160, SONY, NY, USA) during the first 2 h of the scotophase (dark photophase), throughout their lifetime. Flight activity was defined as the cumulative number of seconds the moth was in flight using iMovie (Apple Inc. CA, USA).

Laboratory foraging experiment: feeding behavior, nectar preference, and ambient humidity

The foraging behavior experiments were conducted in 2010–2011. Twenty white 30.5 cm^3 insect observation cages (BioQuip, Rancho Dominguez, CA, USA) were placed in each of two environmental rooms (40 cages total) kept on a 16:8 L:D cycle and at a constant temperature of 28 °C (which is well within one standard deviation of the mean ($\pm$ SD) temperature encountered by moths throughout the field survey period at the SRER: 31.5 $\pm$ 11.4 °C, $n = 10{,}176$). During an experiment, each room was maintained at one of four humidity levels (20, 40, 60 or 80 % RH) using high-capacity humidifiers (Essick MoistAIR Whole House Humidifier HD1407; Little Rock, AR, USA). Experiments at the lowest humidity were conducted during winter when ambient indoor RH was below 20 %. Experiments requiring higher humidity levels were conducted in a randomized block design between fall 2010 and summer 2011. The humidity level in each chamber was monitored every 15 min using U12-012 HOBO data loggers (Onset, Bourne, MA, USA) throughout the experiment and verified that the actual RH was within $\pm$5 % of the experimental RH. For each treatment, we used the following numbers of moths: 20 % RH, $N = 25$; 40 % RH,

$N = 25$; 60 % RH, $N = 27$ and 80 % RH, $N = 22$ with a balanced representation of males and females in each RH treatment.

In the wild, *M. sexta* normally feed from flowers that produce nectar with sugar concentrations between 12 and 22 % w/w (Raguso and Willis 2003; Riffell et al. 2008). In order to situate our nectar sugar manipulations within this natural range, we placed three ad libitum feeders filled with 10 mL of either 0 % (dH$_2$O), 12 or 24 % w/V sucrose solutions (hereafter referred to as "nectar types") in each of the observation cages. The feeders consisted of a 10-mL syringe with an artificial flower made of a white plastic funnel covered on the outside with blue painters tape (Scotch 3M) to provide contrast with the white cages and because *M. sexta* moths have an innate preference for the color blue (Goyret et al. 2008). The funnel was inserted into the open section of the syringe. Moths could easily extend their proboscis and feed from the feeders. Nectar consumption was calculated by reading the scale on the syringe. All feeders were monitored daily. If a feeder's volume fell below 9 mL, then that feeder was replaced with a new feeder filled with 10 mL of the same type of nectar. Newly emerged adult moths were individually placed into 16 (of the 20) observation cages within each environmental chamber. The remaining four cages were used as moth-free controls, set up as were experimental cages but without a moth, so that a baseline of evaporative water loss from each type of feeder could be calculated throughout the experiment. The appropriate controls for each RH treatment and nectar concentration were used to correct for evaporative water loss: if daily intake fell below the average evaporation rate, then intake volume was scored as zero. Moths were observed daily, at least 24 h from the previous observation period, until they were no longer alive. Each day changes in feeder volume (>100 μL) for each of the three feeders were visually measured and recorded in all the cages.

We recorded and calculated the following response variables for each humidity treatment: (1) lifespan in days, (2) average flight activity throughout the experiment, (3) average number of days feeding, (4) proportion of time moths fed during their lifetime, (5) average daily flight activity during scotophase, (6) average volume (μL) of total fluid imbibed daily, (7) average amount of energy (kJ) consumed daily, (8) average volume of total fluid imbibed throughout the experiment, (9) average volume imbibed from specific nectar types throughout the experiment, (10) average amount of energy (kJ) consumed throughout the experiment, and (11) percentage of moths that fed during the experiment. Data on the volume of nectar imbibed were log transformed prior to statistical analysis. Percentage/proportion data were arcsine transformed prior to analysis. As moth lifespan differed significantly among the RH

levels (see below), we normalized the data of the nectar volume imbibed, percent days foraging and daily activity to 50 % lifetime for each RH treatment.

In order to determine the amount of energy (kJ) consumed by a moth, we first calculated the actual amount of sugar imbibed (g) by each individual using the following formula:

$$\text{Actual sugar imbibed} = V_{12\%} * [w/V]_{12\%} + V_{24\%} * [w/V]_{24\%}$$

where V is volume (mL) and $[w/V]$ is the sucrose concentration of each feeder (120 g/880 mL for 12 % sucrose solution and 240 g/760 mL for 24 % sucrose solution). Since 1 g of sugar is equal to 4.2 kcal and 1 kcal is equal to 4.184 kJ, we multiplied the actual amount of sugar imbibed by 17.57 (=4.2 kcal/g sugar × 4.184 kJ/kcal) to obtain the amount of kJ imbibed.

We used simple linear regression analysis in JMP (Version 9) to regress each response variable against RH as an independent variable. To determine if there was an effect of humidity on the proportion of moths foraging from each nectar type, we performed a contingency analysis of nectar type by RH. Average daily activities were compared using the Kruskal–Wallis test (R Development Core Team 2011). Time–event analyses were performed in R using survival statistics, including Cox proportional hazards regression using the R package "survival" (Therneau 2011) and post hoc Tukey multiple comparisons with Bonferroni correction. Repeated measures (RM) ANOVA were made in JMP (Version 8.0.2) and were used to test the significance of the univariate repeated measures factor with four levels. Total flight time was the repeated measure, and treatment was a fixed factor. The *time × treatment* interaction term indicated whether the activity times of *M. sexta* maintained in four different RH environments were affected similarly over time. All statistical analyses were done using all individuals. For clarity, the figures show mean data.

Results

Ambient humidity levels changed dramatically through the monsoon season at the field site (Fig. 1). Humidity levels increased from ∼20 % RH pre-monsoon to ∼80 % RH during the rainy months of July and August. During the entire season (Fig. 1), a total of 205 individual hawkmoths were collected at the MV lights, including 5 large-bodied species [*Agrius cingulata* ($n = 2$), *Hyles lineata* ($n = 148$), *Manduca quinquemaculata* ($n = 15$), *M. rustica* ($n = 1$) and *M. sexta* ($n = 40$)]. With increased humidity, the total number of hawkmoths and the number of *M. sexta* collected at the site also increased (Fig. 1).
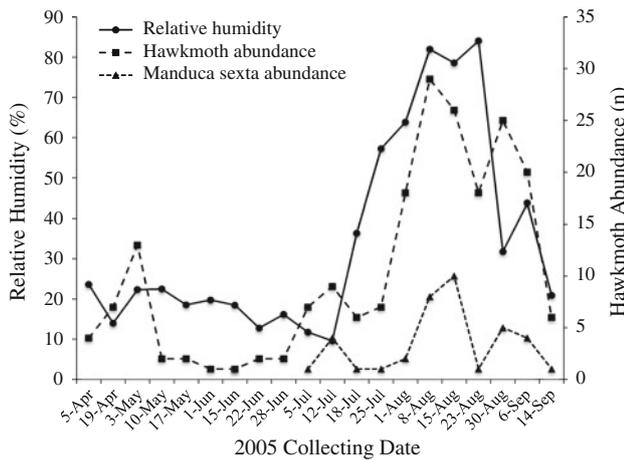
Fig. 1 Changes in ambient relative humidity, hawkmoth abundance, and *Manduca sexta* abundance throughout the 2005 season. The first rainfall event of the 2005 monsoon season occurred on July 16th and the last rainfall event occurred on September 30th

Fig. 2 Percentage of moths alive per day, under four different RH treatments. The *horizontal black dashed line* depicts 50 % survivorship. Sample sizes were 20 % RH, $N = 25$; 40 % RH, $N = 25$; 60 % RH, $N = 27$ and 80 % RH, $N = 22$ with a balanced representation of males and females in each RH treatment

Hawkmoth abundance increased significantly with RH ($F = 23.85$, $df = 18$, $R^2 = 0.57$, $P < 0.0001$) across the duration of the survey (including the pre-monsoon period), but only marginally after the onset of the monsoons ($F = 4.4$, $df = 8$, $R^2 = 0.36$, $P = 0.0692$; Fig. 1). *Manduca sexta* abundance did not increase with RH during the monsoon season measured from the first rainfall event on July 16th 2005 ($F = 1.6$, $df = 8$, $R^2 = 0.19$, $P = 0.2463$). As with hawkmoth abundance, hawkmoth diversity increased significantly with RH ($F = 28.71$, $df = 19$, $R^2 = 0.62$, $P < 0.0001$) across the duration of the survey, but not for the period after the onset of the monsoon only ($F = 0.86$, $df = 8$, $R^2 = 0.097$, $P = 0.3804$).

In our laboratory experiments, we observed differences in the mean lifespan of *M. sexta* moths held at different levels of ambient RH (Fig. 2). In general, moths placed at 20 % RH died earlier (50 % mortality = 7 days) than moths placed at 80 % RH (50 % mortality = 12 days) (Fig. 2, black dashed horizontal line). Therefore, overall moth survivorship increased significantly at higher ambient RH ($F = -23.291$, $df = 98$, $P < 0.0001$). Mean ($\pm$ SEM) survivorship was 6.6 ± 0.6 days at 20 % RH, 8.8 ± 0.9 days at 40 % RH, 12.5 ± 1.3 days at 60 % RH and 12.7 ± 1.1 days at 80 % RH.

Flight activity of *M. sexta* was positively correlated with RH levels ($F = 3.83$, $df = 30$, $R^2 = 0.113$, $P = 0.06$; Fig. 3). However, subsequent pairwise comparisons revealed no significant differences among treatments (Kruskal–Wallis test; Fig. 3). The actual number of days during which moths fed also increased significantly with higher RH ($F = 11.86$, $df = 96$, $R^2 = 0.11$, $P = 0.0009$; Fig. 4a) presumably because moths at higher RH live longer (Fig. 2). However, when these data were
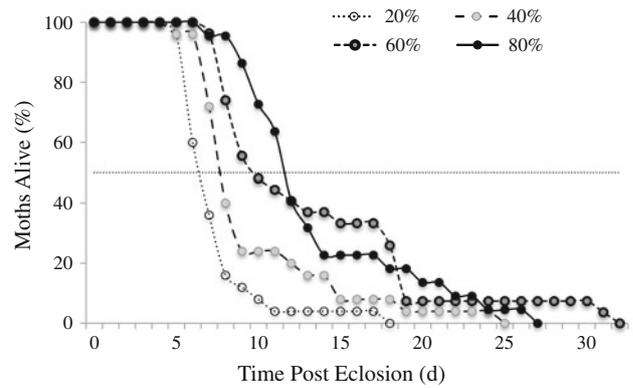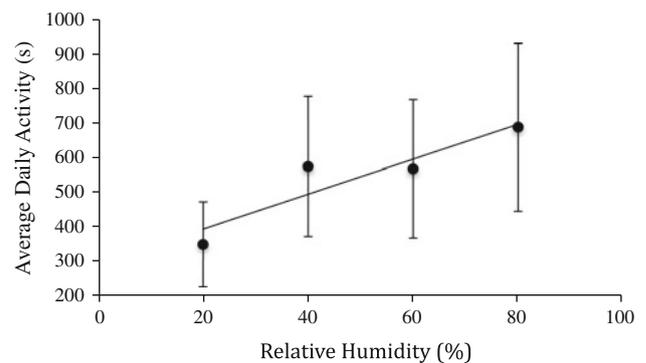

Fig. 3 Average flight activity of *M. sexta* during the first 2 h of scotophase confined to environments with different levels of RH. Average activity level ($N = 8$ per treatment) $\pm$ standard error are shown

standardized by the 50 % survival rate of each treatment, there was a significant negative relationship between RH and the percentage of days foraging, although RH explained only 5 % of the variation ($F = 4.58$, $df = 96$, $R^2 = 0.05$, $P = 0.0350$; Fig. 4b).

Repeated measures ANOVA revealed significant time [$F_{(3/28)} = 3.13$, $P = 0.04$] and treatment [$F_{(11/18)} = 11.7$, $P < 0.0001$] effects in the daily activity patterns of *M. sexta* during the first 2 h of scotophase (Fig. 5). A nonsignificant *time × treatment* interaction indicates that moths kept at different RH levels did not differ in their activity patterns over time [RM ANOVA, $F_{(33/53.7)} = 1.48$, $P = 0.1$; Fig. 6]. Moths showed maximum activity levels 3–4 days after eclosion, regardless of RH treatment (Fig. 5). However, ambient humidity had a significant effect on the age at which maximum average volumes of nectar were consumed ($\chi^2 = 34.46$, $df = 3$, $P < 0.0001$;
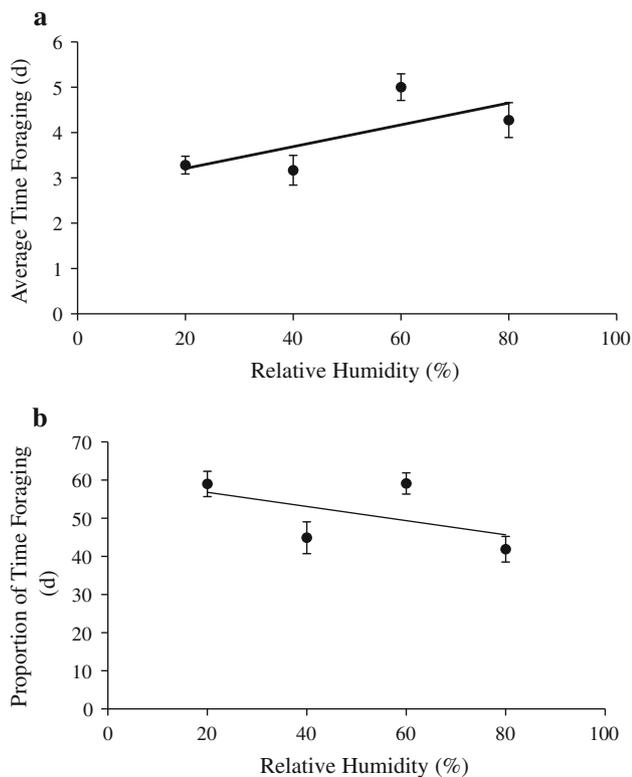
Fig. 4 **a** Effect of RH on the average number of days that moths fed and **b** the proportion of time (days feeding/lifespan) that moths fed
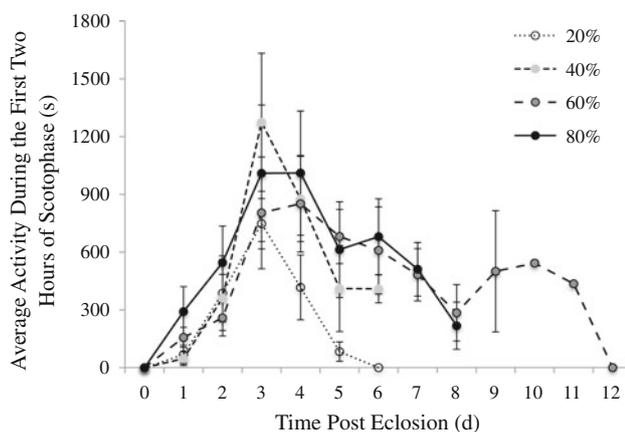

Fig. 5 Temporal pattern of the average (± SEM) flight activity of *Manduca sexta* during the first 2 h of scotophase in different RH treatments ($N = 8$ for all treatments)

Fig. 6a) and at which maximum average energy intake occurred (kJ) (Fig. 6b). At 20 % RH, moths consumed the maximum daily average ($\sim$430 μL) of fluid on the third day after eclosion. This was almost double the maximum daily average volume of fluid consumed by moths placed at 80 % RH ($\sim$230 μL), which was recorded 7 days after eclosion. Although moths consumed a maximum of 1.72 kJ
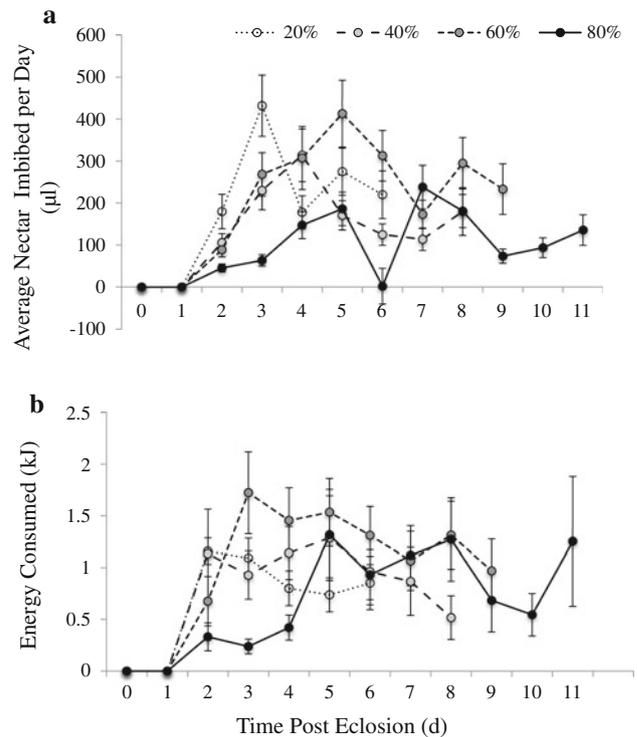

Fig. 6 **a** Average (±SEM) nectar imbibed per day (μl) and **b** daily energy (kJ) consumption by moths in each of the four RH treatments

regardless of humidity treatment, those placed at 20 % RH consumed this amount of energy 3 days sooner than those placed at 80 % RH F = 47.43, $df = 95$, $R^2 = 0.33$, $P < 0.0001$) (Fig. 7).

Mean water evaporation was 200 μL/day for all nectar concentrations at 20 and 40 % RH and between 50 and 70 μL/day for 60 and 80 % RH (all standard errors were ca. 10 μL/day). Over 96 % of moths in each treatment fed during the experiment. The average volume of nectar imbibed per moth decreased significantly with increased RH ($F = 8.11$, $df = 95$, $P = 0.005$; Fig. 8a). When moths foraged, they consumed on average 330 ± 30 μL of nectar at 20 % RH, whereas moths consumed on average 250 ± 40 μL of nectar at 80 % RH. In general, moths consumed less from feeders containing water ($F = 22.74$, $df = 83$, $P < 0.0001$) and 12 % sucrose ($F = 8.12$, $df = 81$, $P = 0.006$) as RH increased (Fig. 8b). However, the volume consumed from the 24 % sucrose solution did not change significantly as RH increased ($F = 0.70$, $df = 84$, $P = 0.41$). Since the actual concentration of sucrose imbibed by moths did not vary significantly as humidity changed, the energy obtained (kJ) by moths throughout the experiment also remained nearly constant among humidity treatments ($F = 2.98$, $df = 95$, $P = 0.09$; Fig. 8c) with an average intake of 0.85 kJ per foraging day.
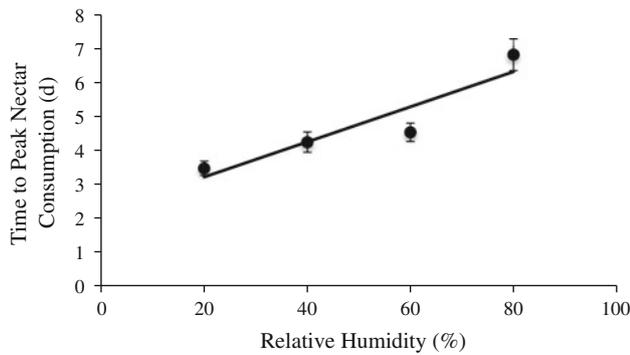
Fig. 7 Differences in the time elapsed (mean days ± SEM) before maximum intake rates by *Manduca sexta* placed in the different RH levels
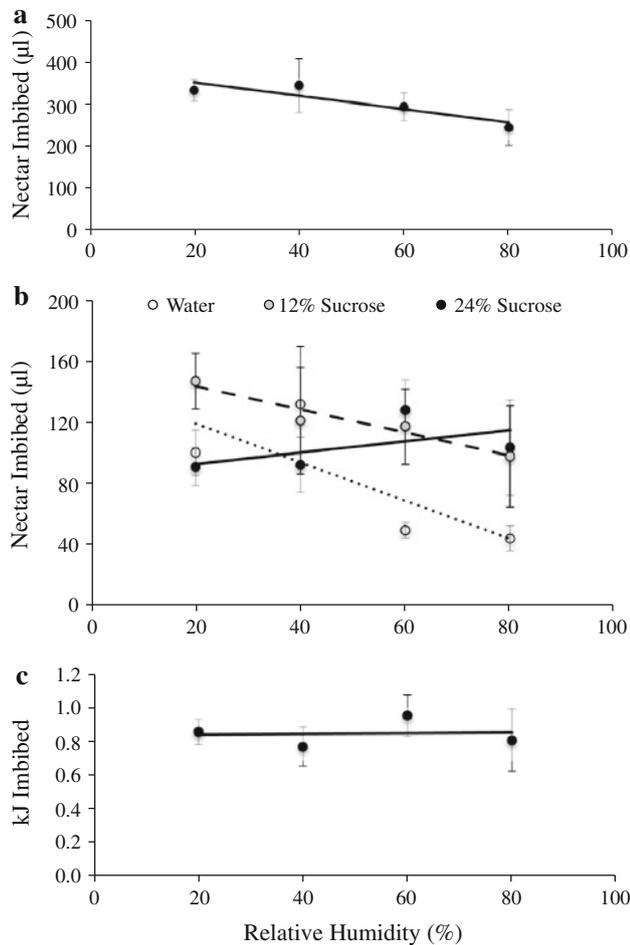

Fig. 8 **a** Average volume of fluid imbibed (μl) per foraging day, regardless of nectar concentration, by moths placed in the four different RH treatments. **b** The average volume imbibed (μl) from specific nectar concentrations and **c** the average amount of energy (kJ) consumed

Discussion

Our field survey confirmed that hawkmoth abundances and diversity increased dramatically with the onset of the

monsoon season (Fig. 1). However, this was true only when the extremely dry pre-monsoon season was included in the analysis, suggesting that it is the qualitative difference in RH before and after the onset of the monsoon season that is most relevant to wild hawkmoths. At different levels of ambient humidity, *M. sexta* modulates its feeding behavior to maintain osmotic homeostasis while maintaining a constant level of energy input (Figs. 6, 8), even though activity levels increased with humidity (Fig. 3). Overall, a larger volume of nectar was imbibed when ambient humidity was low (Fig. 8a), and *M. sexta* preferred to feed from more dilute nectars at these low humidity levels (Fig. 8b). Furthermore, moths showed different daily foraging patterns under different humidity levels (Figs. 6, 7). At higher ambient humidity, moths consumed maximum volumes of nectar and energy at later ages compared to moths that were found in drier conditions (Fig. 7).

Survivorship and activity levels: the effects of humidity

Survivorship (Fig. 2), flight activity (Fig. 3) and the average number of days spent feeding (Fig. 4a) by adult *M. sexta* moths increased with ambient humidity, independent of differences in survival among the RH treatments. Although the average number of days that *M. sexta* fed increased with RH (Fig. 4a), the proportion of time that they fed during the first 50 % of their lifespan decreased with RH (Fig. 4b). This result suggests that moths at lower ambient humidity forage slightly earlier in their lives than do moths at higher RH. Over 96 % of moths were observed to feed on only half of the days of their lifespan. In general, moths were most active during the 3rd–4th day after emergence, regardless of humidity level (Fig. 5). However, moths kept under low humidity showed decreased average daily flight activity during the first 2 h of scotophase, the time of day when nocturnal hawkmoths forage most actively (Gregory 1964; Ramaswamy 1988; Martins and Johnson 2007), compared with moths placed at higher humidity (Fig. 5).

These results are consistent with the increased survivorship under higher ambient humidity observed in other terrestrial insects (Collier and Smith 2010; Yee-Fatt and Chow-Yang 2010; Simmons and Mahroof 2011). It is not completely clear, however, how RH affects activity patterns in insects. In some insects, activity levels increase with increased humidity (Juillet 1964; Kaspari and Weiser 2000; Zhang et al. 2008; Liu et al. 2011) while in others, they decrease (Bursell 1956; Juillet 1964; Barrass 1970; Human et al. 1998). Although insect size plays an important role in determining behaviors that will maintain proper physiological thermal and osmotic levels (Willmer 1986, 1988), it does not appear to explain differences in activity

responses associated with RH (Juillet 1964; Kaspari and Weiser 2000). Instead, differences in activity levels might be explained by an insect's need both to avoid desiccation and to track food availability at different RH (Kaspari and Weiser 2000). In the wild, differences in spatial and temporal distribution of resources restrict insect search activity to the most productive times to ensure that resources are located successfully and efficiently (Bell 1990). Environmental cues, such as time of day, temperature and/or ambient humidity are used to determine not only when the search behavior should be initiated, but also how insects will move through the environment (habitat, patch or food item) to find these resources (Hassell and Southwood 1978; Bell 1990). Adult hawkmoth eclosion (and subsequent increase in hawkmoth abundance) coincides with the onset of the monsoon rains in the northern Sonoran Desert (Fig. 1), during which time floral nectar becomes available (Raguso et al. 2003; Alarcón et al. 2008). For adult *M. sexta*, high humidity may indicate an abundance of critical resources (host plants, nectar sources, available mates), promoting an increase in activity as humidity rises.

Balancing osmotic and energetic demands in different ambient RH

Differences in *M. sexta* survival rates were accompanied by differences in the daily patterns in which moths foraged (Fig. 6). Higher ambient humidity levels increased the age at which a maximum daily average volume of fluid (Fig. 6a) and energy (kJ; Fig. 6b) was imbibed. At 20 % RH, moths consumed maximum volumes of fluid and energy ~2 to 3 days after emerging as adults (Fig. 6), whereas at 80 % RH, moths consumed maximum volumes of fluid and energy ~5 to 7 days after emergence (Fig. 6). Overall, *M. sexta* moths held at lower RH environments imbibed larger volumes (μL) of fluid on average than did moths placed in higher RH environments (Fig. 8a). These differences in fluid intake were due to a decrease in the consumption of the more dilute nectars, water and 12 % sucrose, than when placed in high RH environments (Fig. 8b). The amount of concentrated nectar (24 % sucrose) imbibed, however, remained relatively constant across all RH treatments (Fig. 8b). Although at 20 % RH, moths consumed double the volume of fluid than at 80 % RH, the maximum energy content (kJ) imbibed by moths at these extreme RH treatments did not differ (Fig. 6b). Changes in feeding patterns might be explained by the differences in RH to which these moths were subjected. Moths under low RH conditions consumed dilute (water and 12 % sucrose) nectar much sooner than did those at higher RH, to regulate internal water concentrations. Furthermore, at low RH they consumed a higher volume of these dilute nectars.

Shifting feeding patterns to optimize water balance when ambient conditions promote dehydration has been observed in other insects. For example, the mason bee, *C. sicula* increases the number of flowers visited per foraging trip during hot and dry ambient conditions (Willmer 1986). Furthermore, this bee, like many other insects, switches its preferences from concentrated nectars to more dilute nectars to fulfill their specific water demands (see Watt et al. 1974; Ohguchi and Aoki 1983; Willmer 1986, 1988). When feeding from these dilute nectars, a higher volume of fluid is ingested, compared with when more concentrated nectars are consumed (Lopez-Calleja et al. 1997; Lotz and Nicolson 1999; Martínez del Rio et al. 2001).

Insect flight is energetically costly, and for hawkmoths, which normally hover while feeding, metabolic rates have been shown to increase 100-fold from their resting metabolic rates (Heinrich 1971, 1973; Bartholomew and Casey 1978). Starved moths primarily use lipids to fuel flight (Ziegler and Schulz 1986a, b; Ziegler 1991), whereas recently fed individuals initially utilize imbibed carbohydrates as fuel for flight and switch to stored lipids once carbohydrate stores are depleted (Joos 1987; O'Brien 1999). This switch in substrate use is beneficial: moths realize a roughly 16 % increase in available energy (which can be used for flight) when sugars are oxidized directly, compared with when they are converted to fat prior to oxidation (Suarez et al. 1990). These metabolic considerations suggest that hawkmoths should preferentially consume highly concentrated nectars when flight activity is increased, as a moth weighing 2 g must consume at least 2 mg of sugar per minute to sustain its energetic demand while foraging (Heinrich 1974). In fact, insects that normally exhibit high-energy requirements tend to feed from more concentrated nectars to meet energetic demands (Watt et al. 1974). However, in our study, the amount of energy (kJ) consumed by moths throughout all experimental RH levels was nearly constant: ~0.85 kJ (Fig. 8c) despite the fact that activity was altered with humidity (Fig. 4).

In the Sonoran Desert, wild *M. sexta* moths will readily feed from bat-pollinated *Agave palmeri* flowers (which provide a glucose/fructose rich nectar ~12 % w/w), even though the moths innately prefer flowers of *Datura wrightii*, which provide a sucrose-rich nectar of 21–25 % w/w (Alarcón et al. 2008, 2010; Riffell et al. 2008). Previous studies have suggested that this switch in floral preference is simply a result of flower availability from the preferred plant (Alarcón et al. 2008). However, switching preferences from hexose-rich to sucrose-rich nectars may also be associated with activity level, given that the amount of energy imbibed per unit volume is increased in sucrose-based nectars compared with hexose nectars (Beuchat et al. 1990; Watt et al. 1974). Our results suggest that shifting preferences of *M. sexta* between flowers of *D. wrightii* and

A. *palmeri* might reflect dynamic responses of moths to shifting osmotic demands, an hypothesis that merits further experimental attention. Of course, the different osmolalities of hexose-rich and sucrose-rich nectars (Nicolson 1998; Nicolson and Thornburg 2007) must also be taken into account when determining the nectar preferences of these hawkmoths. Hexose nectars usually have a higher osmolality than sucrose-rich nectars of the same concentration (Corbet 1978; Corbet et al. 1979; Nicolson 1994, 1998). However, in dry environments this relationship is switched, and sucrose-rich nectars actually are more concentrated than hexose-rich nectars due to the high rate of evaporation of sucrose-rich nectars (Corbet et al. 1979; Nicolson 1998). Our field survey indicates that *M. sexta*, and hawkmoths in general, are not active in the very dry period before the onset of the summer rains (it is assumed that the deep penetration into the soil of the first heavy summer rain is the trigger for eclosion, but this has never been tested). Once they become active, however, when humidity is low, *M. sexta* might prefer to feed from the dilute hexose-rich nectar of *A. palmerii*, but switch their preference to the concentrated sucrose-rich nectar of *D. wrightii* flowers when ambient humidity increases—a pattern shown previously in this study system (Alarcón et al. 2008). Although osmotic stress appeared to play a major role in *M. sexta* foraging decisions under our experimental conditions, a detailed study measuring how ambient humidity may affect osmoregulation in *M. sexta* is still needed. Future work should also examine the specific effects of ambient humidity on the flight activity and energetic cost of free-flying moths.

References

Adams DK, Comrie AC (1997) The North American monsoon. B Am Meteorol Soc 78:2197–2213

Alarcón R, Davidowitz G, Bronstein JL (2008) Nectar usage in a southern Arizona hawkmoth community. Ecol Entomol 33:503–509

Alarcón R, Riffell JA, Davidowitz G, Hildebrand JH, Bronstein JL (2010) Sex-dependent variation in the floral preferences of the hawkmoth *Manduca sexta*. Anim Behav 80:289–296

Baker HG, Baker I (1982) Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In: Nitecki MH (ed) Biochemical aspects of evolutionary biology. University of Chicago Press, Chicago, pp 131–171

Baker HG, Baker I (1983) Floral nectar constituents in relation to pollinator type. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 117–141

Barrass R (1970) The activity of *Glossina morsitans* Westwood (Diptera: Muscidae) in laboratory experiments. Proc R Entomol Soc A 45:114–122

Bartholomew GA, Casey TM (1978) Oxygen consumption of moths during rest, pre-flight warm-up and flight in relation to body size and wing morphology. J Exp Biol 76:11–25

Bell WJ (1990) Searching behavior patterns in insects. Annu Rev Entomol 35:447–467

Bell RA, Joachim FG (1976) Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. Ann Entomol Soc Am 69:365–373

Beuchat CA, Calder WA, Braun EJ (1990) The integration of osmoregulation and energy balance in hummingbirds. Physiol Zool 63:1059–1081

Bradley TJ (2009) Terrestrial animals. In: Bradley TJ (ed) Animal osmoregulation. Oxford University Press Inc., New York, pp 111–132

Bradley TJ, Briscoe AD, Brady SG, Contreras HL, Danforth BN, Dudley R, Grimaldi D, Harrison JF, Kaiser JA, Merlin C, Reppert SM, Vanden Brooks JM, Yanoviak SP (2009) Episodes in insect evolution. Integr Comp Biol 49:509–606

Brusca RC, Moore W (2013) A natural history of the Santa Catalina Mountains, Arizona, with an introduction to the Madrean sky islands. Arizona-Sonora Desert Museum Press, Tucson

Bursell E (1956) The effect of humidity on the activity of tsetse flies. J Exp Biol 34:42–51

Butler CG (1940) The choice of drinking water by the honeybee. J Exp Biol 17:253–261

Butler CG (1974) The world of the honeybee. Collins, London

Chown SL (2002) Respiratory water loss in insects. Comp Biochem Physiol A 133:791–804

Collier KJ, Smith BJ (2010) Interactions of adult stoneflies (Plecoptera) with riparian zones I. Effects of air temperatures and humidity on longevity. Aquat Insect 22:275–284

Cooper PD, Schaffer WM, Buchmann SL (1985) Temperature regulation of honey bees (*Apis mellifera*) foraging in the Sonoran desert. J Exp Biol 114:1–15

Corbet SA (1978) Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. Ecol Entomol 3:25–37

Corbet SA, Delfosse ES (1984) Honeybees and the nectar of *Echium plantagineum* L. in southeastern Australia. Aus. J. Ecology 9:125–139

Corbet SA, Willmer PG, Beament JWL, Unwin DM, Prys-Jones OE (1979) Post-secretory determinants of sugar concentration in nectar. Plant Cell Environ 2:293–308

Davidowitz G (2002) Does precipitation variability increase from mesic to xeric biomes? Global Ecol Biogeogr 11:143–154

Davidowitz G, D'Amico LJ, Nijhout HF (2004) The effects of environmental variation on a mechanism that controls insect body size. Evol Ecol Res 6:49–62

Davidowitz G, Nijhout HF, Roff DA (2012) Predicting the response to simultaneous selection: genetic architecture and physiological constraints. Evolution 66:2916–2928

Douglas MWR, Maddox A, Howard K, Reyes S (1993) The Mexican monsoon. J Climate 6:1665–1677

Free JB (1977) The social organization of honey bees. Edward Arnold, London

Goyret J, Pfaff M, Raguso RA, Kelber A (2008) Why do *Manduca sexta* feed from white flowers? Innate and learnt colour preferences in a hawkmoth. Naturwissenschaften 95:569–576

Gregory DP (1964) Hawkmoth pollination in the genus *Oenothera*. Aliso 5:385–419

Haber WA, Frankie GW (1982) Pollination of *Leuhea* (Tiliaceae) in Costa Rican deciduous forest. Ecology 63:1740–1750

Haber WA, Frankie GW (1989) A tropical hawkmoth community: Costa Rican dry forest Sphingidae. Biotropica 21:155–172

Hadley NF (1994) Water relations of terrestrial arthropods. Academic Press, San Diego

Hamilton WJ, Seely MK (1976) Fog basking by the Namib Desert beetle, *Onymacris unguicularis*. Nature 262:284–285

Hassell MP, Southwood TRE (1978) Foraging strategies of insects. Ann Rev Ecol Syst 9:75–98

Heinrich B (1971) Temperature regulation of the sphinx moth, *Manduca sexta*. I. Flight energetics and body temperature during free and tethered flight. J Exp Biol 54:141–152

Heinrich B (1974) Thermoregulation in endothermic insects. Science 185:747–756

Heinrich B, Casey TM (1973) Metabolic rate and endothermy in sphinx moths. J Comp Physiol 82:195–206

Heyneman AJ (1983) Optimal sugar concentrations of floral nectars—dependence on sugar intake efficiency and foraging costs. Oecologia 60:198–213

Hodges RW (1971) The Moths of America north of Mexico. Fascicle 21: Sphingidae. Wedge Entomological Research Foundation

Human KG, Weiss S, Weiss A, Sandler B, Gordon DM (1998) Effects of abiotic factors on the distribution and activity of the invasive argentine ant (Hymenoptera: Formicidae). Pop Ecol 27:822–833

Joos B (1987) Carbohydrate use in the flight muscles of *Manduca sexta* during pre-flight warm-up. J Exp Biol 133:317–327

Josens RB, Farina WM (1997) Selective choice of sucrose solution concentration by the hovering hawk moth *Macroglossum stellatarum*. J Insect Behav 10:651–657

Josens RB, Farina WM (2001) Nectar feeding by the hovering hawk moth *Macroglossum stellatarum*: intake rate as a function of viscosity and concentration of sucrose solutions. J Comp Physiol A 187:661–665

Juillet JA (1964) Influence of weather on flight activity of parasitic hymenoptera. Can J Zool 42:1133–1141

Kaspari M, Weiser MD (2000) Ant activity along moisture gradients in a Neotropical Forest. Biotropica 32:703–711

Kelber A (2003) Sugar preferences and feeding strategies in the hawkmoth *Macroglossum stellatarum*. J Comp Physiol A 189:661–666

Liu Z, McNeil JN, Wu K (2011) Flight mill performance of the Lacewing *Chrysoperla sinica* (Neuroptera: Chrysopidae) as a function of age, temperature and relative humidity. J Econ Entomol 104:94–100

Lopez-Calleja MV, Bozinovic F, Martínez del Rio C (1997) Effects of sugar concentration on hummingbird feeding and energy use. Comp Biochem Physiol A 118:1291–1299

Lotz CN, Nicolson SW (1999) Energy and water balance in the lesser double-collard sunbird (*Nectarinia chalybea*) feeding on different nectar concentrations. J Comp Physiol B 169:2010–2016

Martínez del Rio C, Schondube JE, McWhorter TJ, Herrera LG (2001) Intake response in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences and coevolutionary effects. Am Zool 41:902–915

Martins DJ, Johnson SD (2007) Hawkmoth pollination of aerangoid orchids in Kenya, with special reference to nectar sugar concentration gradients in the floral spurs. Am J Bot 94:650–659

Mittermeier RA, Robles-Gil P, Hoffmann M, Pilgrim JD, Brooks TB, Mittermeier CG, Lamoreux JL, Fonseca GAB (2004) Hotspots revisited: earth's biologically richest and most endangered ecoregions. CEMEX, Mexico City

Nicolson SW (1994) Eucalyptus nectar: production, availability, composition and osmotic consequences for the larva of the eucalyptus nectar fly, *Drosophila flavohirta*. S Afr J Sci 90:59–75

Nicolson SW (1998) The importance of osmosis in nectar secretion and its consumption by insects. Am Zool 38:418–425

Nicolson SW, Thornburg RW (2007) Nectar chemistry. In: Nicolson SW, Nepi M, Pacini W (eds) Nectaries and nectar. Springer, Dordrecht, pp 215–264

O'Brien D (1999) Fuel use in flight and its dependence on nectar feeding in the hawkmoth *Amphion floridensis*. J Exp Biol 202:441–451

O'Donnell MJ (1977) Site of water vapor absorption in the desert cockroach, *Arenivaga investigata*. PNAS USA 74:1757–1760

Ohguchi O, Aoki K (1983) Effects of colony need for water on optimal food choice in honey-bees. Behav Ecol Sociobiol 12:77–84

Pyke GH, Waser NM (1981) The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 12:260–270

R Development Core Team (2011) R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). Available at: http://www.R-project.org

Raguso RA, Willis MA (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. Anim Behav 64:685–695

Raguso RA, Willis MA (2003) Hawkmoth pollination in Arizona's Sonoran Desert: behavioral responses to floral traits. In: Boggs CL, Watt WB, Ehrlich PR (eds) Evolution and ecology taking flight: butterflies as model systems. University of Chicago Press, Chicago, pp 43–65

Raguso RA, Henzel C, Buchmann SL, Nabhan GP (2003) Trumpet flowers of the Sonoran desert: floral biology of *Peniocereus cacti* and sacred *Datura*. Int J Plant Sci 164:877–892

Raguso RA, LeClere AR, Schlumpberger BO (2005) Sensory flexibility in hawkmoth foraging behavior: lessons from *Manduca sexta* and other species. Chem Senses 30:295–296

Ramaswamy SB (1988) Host finding by moths: sensory modalities and behaviours. J Insect Physiol 34:235–249

Riffell JA, Alarcón R, Abrell L, Davidowitz G, Bronstein JL, Hildebrand JG (2008) Behavioral consequences of innate preferences and olfactory learning in hawkmoth–flower interactions. PNAS 105:3404–3409

Simmons AM, Mahroof RM (2011) Response of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to vapor pressure deficit: Oviposition, immature survival and body size. Ann Entomol Soc Am 104:928–934

Suarez RK, Lighton JRB, Moyes CD, Brown GS, Gass CL, Hochachka PW (1990) Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. Proc Natl Acad Sci USA 87:9207–9210

Therneau T (2011) survival: Survival analysis, including penalised likelihood. R package version 2.36-9. Available at: http://cran.r-project.org/web/packages/survival/index.html (original Splus- > R port by Thomas Lumley)

Tuttle JP (2007) The hawk moths of North America: a natural history study of the Sphingidae of the United States and Canada. Wedge Entomological Research Foundation

Unland HE, Houser PR, Shuttleworth WJ, Yang Z (1996) Surface flux measurements and modeling at a semi-arid Sonoran Desert site. Agr Forest Meteorol 82:119–153

Watson AW, Holle R, Lopez RE (1994) Cloud to ground lightning and upper-air patterns during bursts and breaks in the southwest monsoon. Mon Weather Rev 122:1716–1725

Watt WB, Hoch PC, Mills SG (1974) Nectar resource use by *Colias* butterflies. Oecologica 14:353–374

Willmer PG (1986) Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. J Animal Ecol 55:941–962

Willmer PG (1988) The role of insect water balance in pollination ecology: *Xylocopa* and *Calotropis*. Oecologia 76:430–438

Yee-Fatt H, Chow-Yang L (2010) Effects of temperature and humidity on the survival and water loss of *Cimex hemipterus* (Hemiptera: Cimicidae). J Med Entomol 47:987–995

Zhang Y, Wang L, Wu K, Wyckhuys KAG, Heimpel GE (2008) Flight performance of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) under different temperature and humidity regimes. Physiol Ecol 37:301–306

Ziegler R (1991) Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta*. J Comp Physiol B 161:125–131

Ziegler R, Schulz M (1986a) Regulation of lipid metabolism during flight in *Manduca sexta*. J Insect Physiol 32:903–908

Ziegler R, Schulz M (1986b) Regulation of carbohydrate metabolism during flight in *Manduca sexta*. J Insect Physiol 32:997–1001