

Flower tracking in hawkmoths: behavior and energetics

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Summary

As hovering feeders, hawkmoths cope with flower motions by tracking those motions to maintain contact with the nectary. This study examined the tracking, feeding and energetic performance of *Manduca sexta* feeding from flowers moving at varied frequencies and in different directions. In general we found that tracking performance decreased as frequency increased; *M. sexta* tracked flowers moving at 1 Hz best. While feeding rates were highest for stationary flowers, they remained relatively constant for all tested frequencies of flower motion. Calculations of net energy gain showed that

energy expenditure to track flowers is minimal compared to energy intake; therefore, patterns of net energy gain mimicked patterns of feeding rate. The direction effects of flower motion were greater than the frequency effects. While *M. sexta* appeared equally capable of tracking flowers moving in the horizontal and vertical motion axes, they demonstrated poor ability to track flowers moving in the looming axis. Additionally, both feeding rates and net energy gain were lower for looming axis flower motions.

Key words: hawkmoth, flower tracking, feeding rate.

Introduction

Hawkmoths are adept flyers that typically hover, rather than perch, while feeding from flowers. The flowers on which they feed move in response to both natural air currents and those induced by the moth as it hovers. Indeed, the self-generated air flow from the beating wings of a hawkmoth is powerful (Sane and Jacobson, 2006) and capable of inducing flower motions (<http://faculty.washington.edu/danielt/sprayberry06.mov>). Hawkmoths compensate for such flower motions by tracking them through space (Farina et al., 1994; Kern and Varju, 1998). The diurnal hawkmoth *Macroglossum stellatarum* successfully tracks an expanding and contracting disc at frequencies from 0.15–5 Hz (Farina et al., 1994), and similar behavior has been demonstrated in the crepuscular hawkmoth *Manduca sexta* (Moreno et al., 2000). However, the effect of frequency and direction of flower motion on tracking performance is poorly understood, and the impact of flower motions on feeding performance is completely unknown.

Although it is recognized that pollinator feeding performance can impact the fitness of both Lepidoptera and plants (Hill, 1989; Mothershead and Marquis, 2000), the majority of feeding studies have focused on visitation dynamics (Goulson et al., 1998; Miyake and Yahara, 1998) and effects of nectar concentration (Josens and Farina, 2001). Consequently, the effects of flower motion dynamics on plant–pollinator systems are poorly understood. Perching insects might not be challenged by flower motion, but hovering

feeders could experience significant impacts. Feeding from flowers that are difficult to track could increase handling time and/or lower nectar intake. While flower tracking putatively improves feeding performance, hovering flight is metabolically expensive (Willmott and Ellington, 1997), and the maneuvering associated with flower tracking may add considerable costs. This study combines experimental data on tracking motions and feeding rates with a theoretical model to examine the energetic consequences of floral tracking during nectar feeding bouts.

The energetics of flower tracking could have implications for hawkmoth ecology and evolution. Given this, there may be concerns when using results from captive-reared animals to speculate about impacts on the ecology of natural populations. Although previous studies on sensory control of feeding behavior in *M. sexta* have found comparable results for both captive-reared and wild moths (Raguso and Willis, 2002; Raguso and Willis, 2005), feeding rates could differ between the populations. Therefore, we also measured feeding rates of wild *Manduca* to ascertain how well our colony of moths performed.

This study uses both experimental and theoretical techniques to answer four questions. (1) What frequencies and directions of moving flower are best tracked by *Manduca sexta*? (2) How well does *M. sexta* feed from flowers moving at various frequencies and in different directions? (3) Based upon measured tracking and feeding performance and the associated

costs of maneuvering, what are the energetic consequences of flower tracking across tested frequencies and directions? (4) Are feeding rates of colony-reared *M. sexta* comparable to wild populations?

Materials and methods

Animals

Both colony and wild moths were used in these experiments. All lab experiments were conducted using *Manduca sexta* from the University of Washington colony. The ingredients for the colony moths' diet are given in the Appendix. Field experiments on wild *Manduca* were done at the Sonoran Desert Museum's night pollinator garden in Tucson, Arizona for five nights in September 2004. Although field animals could be easily identified as *Manduca*, discriminating between the species *sexta* and *quinquemaculata* was not possible. However, a previous behavioral study on *Manduca sexta* at the Sonoran Desert Museum during the same time of year in 1996–1998 showed very low numbers of *M. quinquemaculata* in light traps (Raguso and Willis, 2005).

Robotic flower experiments: feeding while tracking

This experimental setup was designed to collect 3D motion trajectories of hawkmoths feeding from a moving robotic flower, allowing simultaneous measurement of tracking performance and feeding rates. Moths were flown in a 1.50 m × 0.61 m × 1.83 m flight chamber, which was dimly lit with incandescent under-counter lights strung around the top perimeter. The overall luminance was less than 1 cd m⁻², the lowest resolution of our Gossen Mavolux 5032C light meter (B&H Photo Video). The walls of the chamber were white poster board decorated with green paper leaves to provide visual texture. The floor and the ceiling of the chamber were both black. The only food source in the chamber was a robotic artificial flower. The flower was a lynx-motion 5-axis robotic arm programmed (in MatLab, by N. Jacobs) to move unidimensionally in all three dimensions: horizontal (*H*), vertical (*V*) and looming (*L*). All axes of motion are in the frame of reference of the feeding moth throughout this manuscript (Fig. 1A). A Sony digital high-8 video camera (30 frames s⁻¹) was positioned to record a lateral view of the robotic flower (*V*–*L* plane). One mirror was positioned above the robot and reflected the dorsal view (*H*–*L* plane) onto a mirror placed behind the robot in the lateral plane, so that the single video camera could capture both views. Mirror positions were adjusted so that these two views (the *V*–*L* and *H*–*L* planes) were orthogonal in the spatial region occupied by a feeding moth. Videos of feeding bouts were used to extract 3D information of the flowers' and moths' paths by digitizing orthogonal views using a MatLab program (provided by M. S. Tu). Pixel to centimeter conversions, using an 8 cm rod and a 10 cm cube, were performed on both planes (*V*–*L* and *H*–*L*). The two orthogonal planes were then normalized relative to the *L*-axis coordinate, giving the 3D coordinates of both the moth and the flower for each digitized frame.

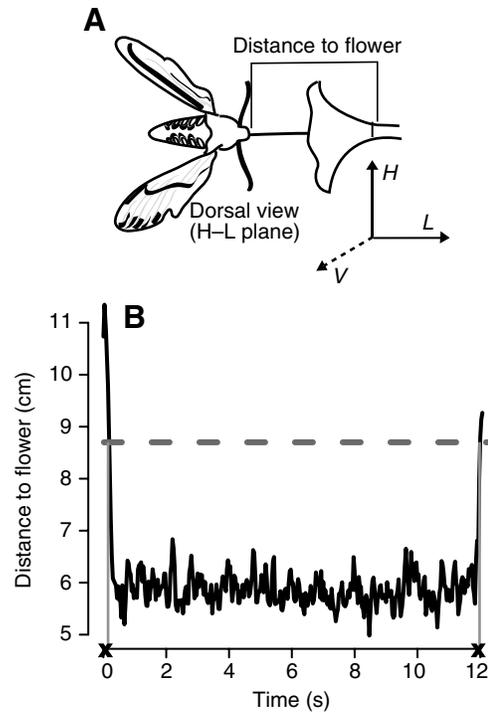


Fig. 1. (A) Line drawing showing the dorsal (mirror) view of a moth feeding from the robotic flower. All axes of motion are defined relative to the moth's perspective. The two axes illustrated are the horizontal axis (*H*), the moth's left and right, and the looming axis (*L*), approach–recede. The vertical axis (*V*, dotted line), is coming out of the page and is up–down for the moth. (B) A schematic showing how we determined the feeding time used for calculating feeding rates. The thick black line shows distance between the moth's head and the base of the flower (*MFD*). The broken line across the graph represents the maximum distance from the flower at which the moth's proboscis could contact nectar (calculated based on average proboscis length of the colony). Feed time was defined as (the last time *MFD* < broken line) – (the first time *MFD* < broken line). The feeding rates were calculated as (amount of nectar consumed)/(feed time).

The flower was constructed of five paper petals in a 'trumpet flower' morphology, similar to hawkmoth-pollinated *Datura* flowers, with a diameter of 5.7 cm. The paper petals were attached to a standard blue 1000 µl pipette tip that allowed easy exchange of standard yellow 200 µl micropipette tips containing nectar. The yellow micropipette tips were filled with 40 µl of 27% sucrose solution. The percentage of sucrose used was in the range of concentrations found in hawkmoth-pollinated flowers [19%–37% (Raguso et al., 2003)]. Likewise, the nectar volume used to measure feeding rates was in the range of volumes found in hawkmoth-pollinated flowers [3–80 µl (Raguso et al., 2003)].

Hawkmoths were subjected to two different experimental treatments that varied frequency (0–3 Hz) and direction (*H*, *V* and *L* axes). We collected video and nectar consumption data of moths feeding from a flower moving at 1, 2 and 3 Hz in the *H* (left–right) and *V* (up–down) axes. Data were collected only for 1 and 2 Hz in the *L* (approach–recede) axis because moths

appeared unable to feed from a flower moving in the L axis at 3 Hz. Five samples were collected for the H axis at 1 and 2 Hz, the V axis at 1, 2 and 3 Hz, and the L axis at 1 Hz. Three samples were collected for the H axis at 3 Hz treatment, and four samples were collected for the L axis at 2 Hz treatment. The amplitude of flower motion was held constant at 1 cm. For horizontally and vertically moving flowers, this was an 8.7° visual displacement. For looming flowers, 1 cm amplitude is equivalent to 7.4° of expansion. Five control events (moths feeding from a stationary flower) were also collected.

Measurement of feeding rates in the field

To assess feeding rates of wild *Manduca*, we monitored feeding activity on artificial flowers with a known nectar volume at the Sonoran Desert Museum. The artificial flowers were identical to those used in laboratory studies and had a mean nectar volume of $42.1 \pm 0.99 \mu\text{l}$. Artificial flowers were arranged around a single *Datura wrightii* bloom to attract moths. The artificial flowers were visited by one wild moth on each of three nights. A single moth visitor would completely drain the nectar reservoir of a 'flower', and typically empty all available artificial flowers. This gave us multiple feeding measurements from three different *Manduca*.

Feeding activity on artificial flowers was filmed (Sony miniDV camcorder, 30 frames s^{-1}) under infrared lighting conditions to avoid disrupting natural light levels (White et al., 2003). Feeding rates were calculated as (nectar volume – evaporative volume)/visit time. As all feeding events resulted in a completely emptied nectar tube, the nectar volume consumed was $42.1 \mu\text{l}$. The evaporative volume was measured from control flowers. The controls had identical nectar volumes and flower morphology, but with black petals to hide them from visitors. They were corked at the end of the feeding session and later weighed to determine evaporative loss. The mean evaporation of control nectar volumes during filming for three nights spent in the field was used as the evaporative volume in feeding rate calculations ($1.9 \mu\text{l}$). Visit time was calculated (using digitized video) by taking the number of video frames a moth's proboscis was seen to be in the flower divided by the frame rate (30 frames s^{-1}).

Measurement of feeding rates in the lab

Nectar consumption was measured by weighing nectar tubes before and after feeding bouts, then calculating nectar consumed as the mass difference between the two. Assuming a density of 1, the nectar mass was converted into volume (μl). The evaporative loss of nectar from tubes over the course of an experiment was insignificant ($1\text{--}3.6 \mu\text{l}$, Student's t -test, $P=0.948$). Feeding rate was defined as (volume of nectar consumed)/(visit time). Visit time was determined from digitized video data. Start time was set as the first time that the moth's distance to the nectary was less than average proboscis length of the colony (8.7 cm). Stop time was set as the last time in a digitized feeding bout the moth's distance to the flower was less than the average proboscis length. Visit time was simply calculated as stop minus start (Fig. 1B).

Analysis of tracking performance

Using digitized data, we measured the mean distance between the hawkmoth's head and the flower for all feeding bouts. These calculations excluded the moth's approach and departure from the flower. The digitized data were also used to compute the cross-correlation of the moth's and flower's spatial trajectories, which provides two metrics of tracking performance: (1) the cross correlation coefficient (r), which accounts for how well a hawkmoth mimics the flower's path, and (2) the lag of behavioral response, which can be used to calculate phase. The cross-correlation coefficient r measures how well the flower's path predicts the moth's path, i.e. how well the moth is tracking the flower. The latency of response is the first time step where r peaks, giving the delay at which correlation between the two signals is maximal (see Results). The phase of moth response is taken as (latency)/(period of the flower motion). Prior to cross-correlation analysis, spatial trajectories of both the moth and flower are put through a first-order, low-pass, Butterworth filter using the 'filtfilt' function in Matlab (providing phase free filtering) to remove high frequency digitizing error. The corner frequency for this filter was 28.4 Hz. Additionally, any slow linear trends in the moths' trajectories are removed by detrending the data using the 'detrend' function in MatLab.

Modeling energy gain

To track moving flowers, moths must necessarily accelerate and decelerate their body mass. As frequency and amplitude of flower motion increase, the rates of acceleration and deceleration will also increase, thereby changing the energetic cost of flight (\dot{E}_{out}). At the same time, any changes in nectar uptake that occur as a result of changing flower dynamics will alter the influx of energy (\dot{E}_{in}). To understand the energetic consequences of tracking we modeled the rate of net energy gain (\dot{E}_{gain}), which is simply the difference between the rates of energy intake and energy output:

$$\dot{E}_{\text{gain}} = \dot{E}_{\text{in}} - \dot{E}_{\text{out}} \quad (1)$$

The rate of energy intake is a linear function of the sucrose concentration, the energy content of sucrose, and the mass rate of nectar uptake:

$$\dot{E}_{\text{in}} = \epsilon f[S], \quad (2)$$

where $[S]$ is the sucrose concentration (0.27), ϵ is the energy content of sucrose ($1.54 \times 10^4 \text{ J g}^{-1}$) (Kingsolver and Daniel, 1979), and f is the mass rate of nectar uptake in g s^{-1} , calculated from the measured feeding rate from each trial.

We modeled the flight costs (rate of energy expenditure) as a linear sum of the rate of energy expenditure required to hover (\dot{E}_{hover}) plus the energy required to track a moving flower (\dot{E}_{track}). Resting metabolic rate was ignored because it is small compared to overall energy expenditures (Kingsolver and Daniel, 1979; Willmott and Ellington, 1997). Because of metabolic inefficiencies, the animal needs to ingest more energy than the mechanical power required to move. Ellington (Ellington, 1984) calculated that muscle efficiency for sphingid

moths ranges from a minimum of 6% (assuming perfect elastic storage of inertial energy) to a maximum of 17% (assuming muscles accelerate the wings without aid of elastic storage). For this model we assigned muscle efficiency (η) to be the mean of these two estimates, 11.5%:

$$\dot{E}_{\text{out}} = (1/\eta)(\dot{E}_{\text{hover}} + \dot{E}_{\text{track}}). \quad (3)$$

Mechanical hovering costs (\dot{E}_{hover}) were presumed constant for a moth of given mass and estimated (Willmott and Ellington, 1997) to be 30 W kg^{-1} . The instantaneous cost of tracking (\dot{E}_{track}) was taken to be the power required to move the body mass as it tracks flower position:

$$\dot{E}_{\text{track}} = ma(t)v(t), \quad (4)$$

where m is mass, $a(t)$ is acceleration and $v(t)$ is velocity of the moth. Because moths were tracking flowers moving as periodic functions, we modeled moth position as a simple periodic function:

$$x(t) = A\sin(2\pi ft). \quad (5)$$

Thus:

$$v(t) = \dot{x}(t) = A2\pi f\cos(2\pi ft) \quad (6)$$

$$a(t) = \ddot{x}(t) = -A(2\pi f)^2\sin(2\pi ft). \quad (7)$$

Substituting Eqn 6 and Eqn 7 into Eqn 4 gives:

$$\dot{E}_{\text{track}} = -mA^2(2\pi f)^3\sin(2\pi ft)\cos(2\pi ft). \quad (8)$$

Since energy is expended in both directions within an axis of motion (represented in a periodic position function as positive and negative), we calculate total energy as the absolute value of Eqn 8. Because that rectified function is periodic at four times the driving frequency, the average of the function is taken over a quarter cycle:

$$\dot{E}_{\text{track}} = mA^2(2\pi f)^3 1/(4f) \int_0^{1/(4f)} \sin(2\pi ft)\cos(2\pi ft)dt = 8m\pi^2 f^3 A^2. \quad (9)$$

We obtained the frequency and amplitude values to calculate power by performing a fast fourier transform (FFT) analysis on the moth's path through space in all dimensions. To remove high frequency digitizing error, the digitized data were filtered as described in above 'Analysis of tracking performance'. The mean was also removed from the signal prior to running the FFT analysis. Removing the mean created an artificial peak by forcing the origin value to zero. Discarding this artifact, we used the top three remaining peaks from the FFT analysis (see Results). The total power output for an individual axis was taken as the summed power output calculated from the individual peaks. Since tracking a flower necessarily involves motion in all three axes, even if the flower only moves in one, we sum the power output components of each axis to predict total tracking cost.

$$\dot{E}_{\text{totaltrack}} = 8m\pi^2 \sum_{j=1}^3 \sum_{i=1}^3 f_{i,j}^3 A_{i,j}^2, \quad (10)$$

where $j=1, 2, 3$ corresponds to the horizontal, vertical and

looming motion axes, and $i=1, 2, 3$ corresponds to the frequency and amplitude peaks in each motion axis.

Results

M. sexta were able to feed from flowers moving at frequencies of 0, 1, 2 and 3 Hz for both the *H* (left–right) and *V* (up–down) axes. However, they were never successful at feeding from 3 Hz flowers in the *L* axis (approach–recede). Consequently, we only have data for 1 and 2 Hz in the *L* axis.

Feeding rates

Feeding rates were relatively independent of frequency, but do show direction effects (Fig. 2). The empty data point caused by moths being unable to feed from *L* axis 3 Hz flowers makes it difficult to run a standard ANOVA on the entire dataset. When analyzing individual axes, only the *L* motion axis showed a weak effect of frequency ($P=0.09$, ANOVA). Additionally, we ran an ANOVA on: (1) control, *H* and *V* axes through 3 Hz; (2) control, *H*, *V* and *L* axes through 2 Hz; (3) *H* and *V* axes at 1, 2 and 3 Hz; (4) *H*, *V* and *L* axes at 1 and 2 Hz. All these analyses returned the same basic findings. There is no effect of frequency on feeding rate. However, there are several direction effects. The *V* axis returns as statistically distinct from control flowers in analyses 1 ($P<0.05$) and 2 ($P<0.01$). The *L* axis returns as statistically distinct from the *H* axis in analyses 2 ($P<0.05$) and 4 ($P<0.05$), and distinct from the control in analysis 1 ($P<0.05$).

Feeding rates of wild *Manduca* were measured to determine if colony moth feeding performance was comparable to hawkmoths in the field. Data were collected from three wild *Manduca* feeding on stationary artificial flowers. The mean feeding rates for these animals were $3.5, 6.6$ and $9.6 \mu\text{l s}^{-1}$, as compared to control data collected in the lab ($4.62, 6.75, 8.08, 8.42$ and $9.71 \mu\text{l s}^{-1}$). Feeding rates of wild *Manduca* and

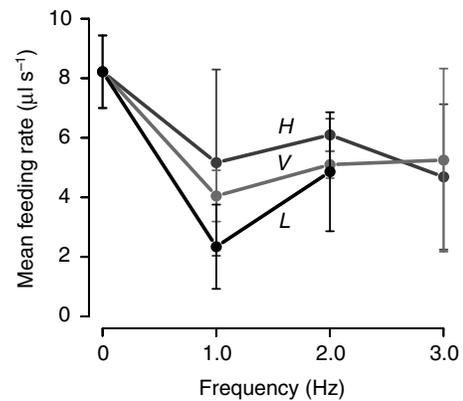


Fig. 2. The mean feeding rate across frequency and direction; the lines for different direction treatments are labeled (*H*, horizontal; *V*, vertical; *L*, looming). Feeding rates measured from moths tracking flowers moving in the *L* axis (approach–recede) are significantly lower than those for control flowers (ANOVA, Tukey's HSD $P<0.05$), but there were no frequency effects when using a multifactor ANOVA.

Table 1. Mean feeding distance

	Distance (cm)			
	Control	1 Hz	2 Hz	3 Hz
Horizontal	6.96±0.17	6.37±0.18	6.35±0.10	6.64±0.17
Vertical	6.96±0.17	6.46±0.05	6.84±0.13	7.15±0.13
Looming	6.96±0.17	6.31±0.15	9.36±0.75	–

Measurements of the mean distance between a feeding moth and the flower remained fairly constant for all feeding bouts.

colony *M. sexta* are statistically indistinguishable from each other (Student's *t*-test, $P=0.662$).

Tracking performance

If the hawkmoths were actively tracking moving flowers, they should maintain a constant distance to a flower while feeding. The mean distance was calculated from digitized data for all feeding bouts (Table 1). Mean distance typically remains constant across frequency; both the *H* and *V* axes showed no effect of frequency. However, the *L* axis did show a significant increase in mean distance with increasing frequency (ANOVA, $P<0.05$). Additionally, an ANOVA on all data showed no frequency effects, but a significant direction effect (*H* versus *L*, $P<0.05$). If moths were successfully tracking flowers, the measurements of moth to flower distance during a feeding bout should have low variance. Indeed we see very low variance for all treatments, with the exception of moths feeding from looming flowers moving at 2 Hz (Fig. 3). Here, the increase in distance seen in Table 1 is accompanied by significant increase in variance ($P<0.05$, ANOVA).

Cross correlation of the digitized flower and moth paths provided a measurement of how well a moth followed a flower, giving both a maximum correlation coefficient that indicates how well a moth mimicked the flower's path (r_m) and the latency of the moth's response. The response latency is

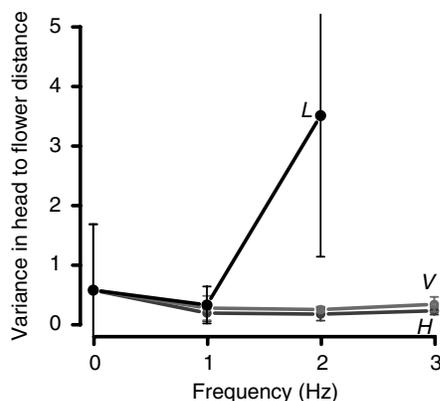


Fig. 3. The variance of flower to moth distance measurements during feeding bouts. The lines for different direction treatments are labeled (*H*, *V* and *L*). The variance for the *L* direction 2 Hz treatment was significantly higher ($P<0.05$, ANOVA, Tukey's HSD).

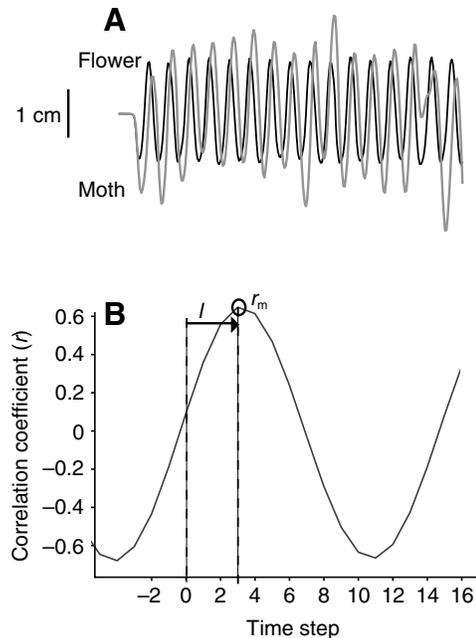


Fig. 4. (A) Sample input data of a moth (gray line) following a flower (black line) in the *V* axis (up-down) at 2 Hz. (B) The resulting cross correlogram: the values for r_m and latency (l) are taken from the circled peak, the first peak after zero.

determined from the time lag associated with the maximum cross-correlation value (r_m) (Fig. 4). The closer r_m is to one, the better the moth is tracking the flower. When comparing the mean r_m values across treatments, there was a significant decrease with increasing frequency in all motion axes ($P<0.01$, ANOVA; Fig. 5). This decrease was not linear for the *H* and *V* axes; rather mean r_m values for both 1 Hz and 2 Hz flowers were statistically indistinguishable, then exhibited a sharp decline at 3 Hz. Likewise, the r_m values for the *L* axis showed a sharp drop at 2 Hz. When looking at both latency and phase of hawkmoth response to moving flowers, there were strong frequency and direction effects. In all motion axes, both latency and phase of response increased with frequency ($P<0.001$, ANOVA; Fig. 6). Additionally, the latency of response to *V* axis flowers is significantly larger ($P<0.001$, ANOVA). Accounting for all data, *M. sexta* seem to track low frequency *H* axis flowers the best, and are poorest at tracking high frequency *L* axis flowers.

Energetics of flower tracking

To ascertain whether or not the energy expended to track flowers has a significant impact on energy gained, we calculated the net rate of energy gain (\dot{E}_{gain}) for each feeding bout. Rate of energy gain was simply modeled as the rate of energy in minus the rate of energy out ($\dot{E}_{\text{in}} - \dot{E}_{\text{out}}$; Eqn 1). Calculations of energy input were made using measured feeding rates. Calculations of energy output utilized coefficients from an FFT analysis of moth position vectors (see Materials and methods). We used the top three peaks from the

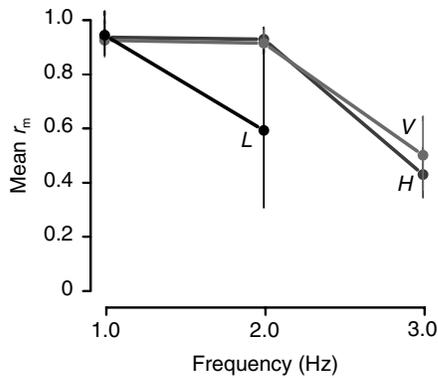


Fig. 5. The mean cross correlation coefficient, r_m , for all treatments. Frequency is on the x axis, and the lines for different direction treatments are labeled (H , V , L). Mean r_m values for tracking ability start to fall off at 2 Hz for moths feeding from flowers moving in the L axis. Moths were unable to track 3 Hz L axis flowers. Both H and V axes flowers have high correlation coefficients for 1 and 2 Hz.

FFT analysis (Fig. 7), the results of which are summarized in Table 2. Flower motion did indeed increase the amount of energy consumed during a feeding bout. However, this had minimal impact on the net rate of energy gain (\dot{E}_{gain}), because

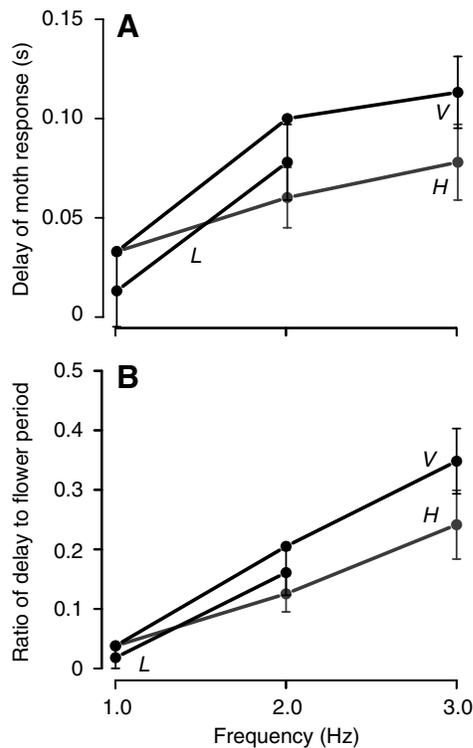


Fig. 6. The delay characteristics of *M. sexta*'s flower tracking ability. (A) Latency of response plotted against frequency. (B) Phase of response plotted against frequency, where phase is defined as (latency of response)/(period of flower motion). A value of zero indicates that the moth is perfectly in phase with the flower, while a value of 0.5 indicates the moth is 180° out of phase. The direction treatments are labeled (H , V , L).

the magnitude of energy out (\dot{E}_{out}) is minute (2–3%) relative to the energy in (\dot{E}_{in}) (Fig. 8).

Discussion

Tracking performance

As hovering feeders, hawkmoths must inevitably contend with flower motion. Such motions arise from both natural airflows and those induced by beating wings (Sane and Jacobson, 2006) (<http://faculty.washington.edu/danielt/sprayberry06.mov>). Hawkmoths compensate for these motions by exhibiting a skilled tracking behavior, maintaining a constant distance to a moving nectary (Table 1). Although *M. sexta* are indeed talented at tracking moving flowers, their ability to do so varies with both frequency and direction (Figs 5, 6). We found that *M. sexta* were best able to track flowers moving with a frequency of 1 Hz. While moths accurately mimicked the flower path for the 2 Hz treatment in the H and V axes, they were farther out of phase with flower motion than moths feeding from flowers in the 1 Hz treatment. *M. sexta* were easily able to track flowers moving in the horizontal and vertical motion

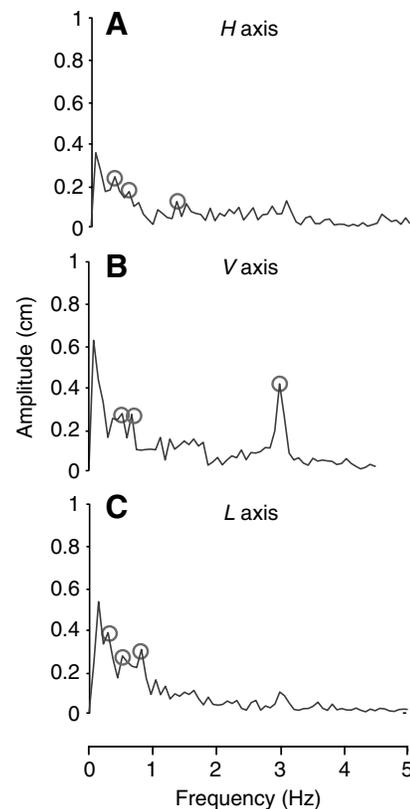


Fig. 7. The results of an FFT analysis on one feeding bout (direction= V axis; frequency=3 Hz). This is how the frequency and amplitude coefficients for calculating rate of energy gain were obtained. Discarding the first peak, which is an artifact due to removing the mean position (see Materials and methods), the remaining three highest peaks are the coefficients used (all included peaks are circled). (A) shows the FFT of moth position for the H axis, (B) for the V axis, and (C) for the L axis.

Table 2. The means of measured FFT coefficients used in calculating power required to move

Coefficient	Control	H1	H2	H3	V1	V2	V3	L1	L2
<i>Freq_{H1}</i>	0.80	1.00	1.99	3.04	0.46	1.44	1.00	0.74	0.90
<i>Amp_{H1}</i>	0.16	0.97	1.30	0.55	0.19	0.25	0.19	0.22	0.57
<i>Freq_{H2}</i>	1.50	1.21	0.53	0.36	1.24	0.95	1.00	1.18	0.80
<i>Amp_{H2}</i>	0.14	0.19	0.27	0.36	0.14	0.21	0.16	0.18	0.35
<i>Freq_{H3}</i>	1.99	1.18	0.83	0.64	0.88	0.70	1.62	0.86	1.37
<i>Amp_{H3}</i>	0.12	0.14	0.18	0.15	0.12	0.19	0.14	0.39	0.31
<i>Freq_{V1}</i>	0.42	0.78	1.18	0.70	1.02	1.99	3.03	0.86	0.80
<i>Amp_{V1}</i>	0.19	0.18	0.17	0.18	1.17	1.45	0.47	0.45	0.71
<i>Freq_{V2}</i>	1.12	1.35	2.05	1.48	1.01	0.41	0.45	0.36	1.53
<i>Amp_{V2}</i>	0.16	0.14	0.11	0.09	0.19	0.29	0.29	0.21	0.44
<i>Freq_{V3}</i>	1.74	1.30	1.79	1.72	2.07	1.46	0.59	1.41	1.20
<i>Amp_{V3}</i>	0.12	0.10	0.09	0.08	0.18	0.20	0.24	0.16	0.33
<i>Freq_{L1}</i>	1.23	0.67	1.63	1.18	0.59	0.89	0.73	1.01	1.99
<i>Amp_{L1}</i>	0.23	0.33	0.44	0.38	0.35	0.39	0.42	1.11	1.69
<i>Freq_{L2}</i>	1.30	0.96	1.47	2.27	0.90	1.14	0.73	0.42	0.20
<i>Amp_{L2}</i>	0.19	0.21	0.17	0.29	0.27	0.33	0.31	0.22	0.85
<i>Freq_{L3}</i>	2.30	1.43	2.57	1.25	1.73	1.21	0.87	0.71	0.81
<i>Freq_{L3}</i>	0.15	0.16	0.11	0.21	0.18	0.27	0.27	0.17	0.65

For each feeding bout, the moth's path was subjected to an FFT analysis. The top three amplitude (Amp) peaks for each axis of motion (*H*, *V* and *L*) were used, along with their corresponding frequencies (Freq). The columns show the different frequency and direction treatments, while the rows are the mean FFT coefficients.

axes. However, they had a markedly lower ability to track flowers moving in the *L* axis. In fact, *M. sexta*'s tracking ability in this direction declined sharply as frequency increased, so that they were unable to feed from a flower moving at 3 Hz.

Previous work on flower tracking in the hawkmoth *Macroglossum stellatarum* stated they were able to track 0.5–5 Hz stimuli in the looming axis (no other directions were studied) (Farina et al., 1994). These results are different than those presented in this paper, but to some extent this may be a result of methodological differences. First, this study used a mechanical stimulus in ramp experiments, moving the flower at a constant velocity of 3 cm s⁻¹ (a speed that would roughly correlate with a 1.5 Hz stimulus in the paradigm of our experiments). However, when investigating the range of frequencies *M. stellatarum* was able to track, Farina et al. used the purely visual stimulus of a projected expanding and contracting disc (Farina et al., 1994). As frequency increased above 3 Hz, the phase of moth response increased dramatically, from trailing 20° out of phase at 3 Hz to approximately 115° at 5 Hz. The highest phase delay at which *M. sexta* were capable of tracking a mechanical looming flower was 109°. It is possible that *M. stellatarum* would not have been able to feed from a mechanical flower oscillating at 5 Hz. However, the phase differential exhibited by *M. sexta* while tracking horizontal and vertical 3 Hz flowers (85–123°) is much larger than *M. stellatarum* tracking 3 and 4 Hz stimuli (15–50°), indicating that *M. stellatarum* can indeed track faster moving flowers.

There are several potential reasons for this difference. Hawkmoth visual systems display changes in tuning of motion-processing that are matched to their ecology (O'Carroll et al., 1997), and *M. stellatarum* are diurnal and active in much

brighter light regimes than *M. sexta*. Given the higher light levels, *M. stellatarum*'s visual system is likely better at resolving faster moving flowers. *M. stellatarum* have higher spatial resolution (1.8°) (Warrant et al., 1999) than *M. sexta* (3°; E. Warrant, personal communication) in their frontal field of view. Additionally, *M. stellatarum* has a much shorter proboscis (2.4 cm) (Krenn, 1990) than *M. sexta* (8.7 cm). At this shorter distance their higher angular resolution results in a much finer cartesian resolution (0.07 cm for *M. stellatarum* versus 0.46 cm for *M. sexta*). However, recent experiments on object-sensitive descending neurons in *M. sexta* show that their visual system responds to an object oscillating at frequencies as high as 6 Hz (J.D.H.S., manuscript in preparation). If vision is not responsible for the roll off in *M. sexta*'s tracking ability, it is less likely that the difference in tracking ability between the two species is attributable to the difference in tuning of their visual systems. In fact, in Farina et al.'s study (Farina et al., 1994) *M. stellatarum* were clearly trying to track frequencies they were incapable of physically following, indicating that their visual inputs are trying to drive motions that the musculoskeletal system is ultimately unable to produce. It is possible that the aerodynamic maneuverability of hawkmoths is limiting their tracking behavior, and *M. stellatarum*'s smaller size contributes to greater maneuverability, allowing them to track flowers at higher frequencies.

Feeding performance

Flower tracking performance is likely to be vital in allowing a moth to maintain contact with a nectar source. For example, the hawkmoth-pollinated flowers *Nicotinia glauca* and *Nicotinia glauca* have corolla widths of 0.6–0.7 cm (Ippolito et al.,

2004) and nectar volumes of 5.82–9.43 μl (Kaczorowski et al., 2005), and thus the depth of the nectar reservoir will range between 0.015 and 0.034 cm. Even a slight shift of the proboscis during feeding will result in loss of contact with the nectar. This will either increase the time the moth has to stay at the flower to drain all of the nectar, or cause them to acquire less than a full nectar load from that visit. Flower tracking should mitigate these effects by minimizing loss of contact with the nectar reservoir. Interestingly, feeding rates do not seem to be tightly correlated with tracking ability. In fact, hawkmoths feed equally well from poorly tracked flowers moving at 3 Hz in the H and V axis as they do from easily tracked flowers

moving at 2 Hz, indicating that suboptimal tracking is still sufficient for maintaining nectar contact. However, the fact that *M. sexta* are completely unable to feed from flowers moving at 3 Hz in the looming axis indicate that some base level of flower tracking is necessary. Flower tracking, then, seems to have a threshold effect on feeding rates; either the moth tracks sufficiently to feed well, or it cannot feed from the flower at all.

Even though feeding rates remain constant, the energetic cost associated with tracking moving flowers should increase as the cube of frequency and as the square of amplitude. We explored the consequences of this by constructing a model of net energy gain using measured inputs of nectar uptake and physical work performed while feeding. We do indeed find that tracking costs rise (Fig. 8), but these cost increases are low relative to the rate of energy intake and do not impact net energy gain on the scale of an individual feeding bout. The hawkmoth *Macroglossum stellatarum* can consume 211 μl of nectar in a day (Kelber, 2003), but even on this scale the 0.4% increase in tracking cost seen between control and 2 Hz flowers results in a loss of only 0.86 μl of nectar. It is unlikely that the energetic cost of tracking flowers is of much significance. However, the effects of direction of flower motion have a stronger impact than frequency on net energy gain. Moths display significantly lower feeding rates when tracking flowers moving in the looming axis, but this tracking costs as much, or more, than tracking flowers moving horizontally or vertically.

Potential evolutionary implications

Considering the tight coevolution between plants and pollinators, surprisingly little attention has been paid to the vibrational properties of flowers. Although Etnier and Vogel (Etnier and Vogel, 2000) suggest torsional mechanics of flowers play an important role in their ability to withstand wind forces, there was relatively little discussion of the dynamics of that motion. However, torsional motions could play a central role in plant–pollinator interactions. Torsional properties of stems could convert what would be poorly tracked L axis motions into more easily tracked lateral, or H axis, motions. Unfortunately, current studies on torsional mechanics of flowers have been phylogenetically limited, and do not include hawkmoth pollinated flowers. The resonant motion dynamics of flowers are impacted not only by environmental stimuli (such as wind), but also by their biomechanical properties. In fact, it is reasonable to assume that the frequency of flower motion scales with the square root of the stem's flexural stiffness (Timoshenko et al., 1974). Additionally, flowers are capable of modifying stem stiffness during development by changing their allocation of biomass (Niklas, 1998), indicating there is some control over biomechanical properties of stems. As such, there is potential for an evolutionary response of hawkmoth-pollinated flowers to hawkmoth tracking performance.

The extent to which there is coevolution between the visual-motor systems of pollinating insects and the mechanical properties of the plants that they pollinate remains unexplored. Recent work (Goyret and Raguso, 2006) shows that

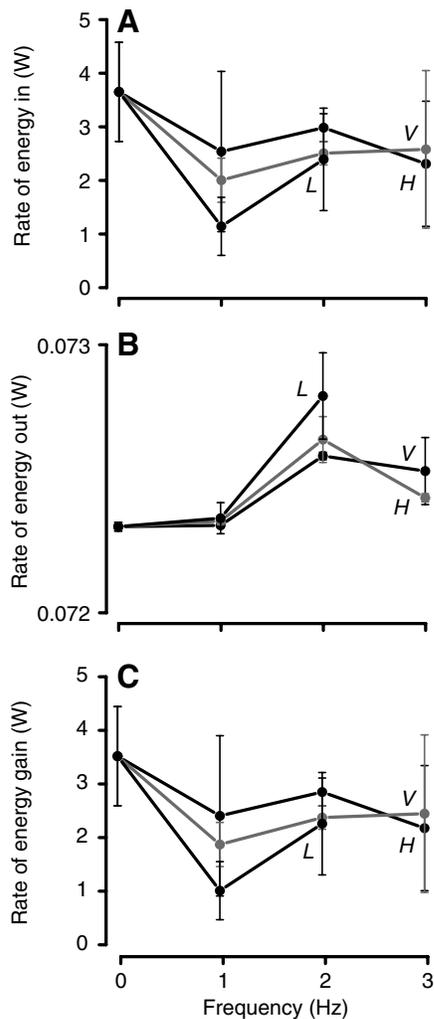


Fig. 8. The results of an energetics model that used flight path and feeding data to calculate energy gain for all treatments. Direction treatments are plotted as separate, labeled lines (H , V , L), while frequency is shown on the x -axis of the plots. Values are means \pm s.e.m. (A) Rate of energy in, calculated using measured feeding rates. (B) Rate of energy out, calculated using coefficients from an FFT analysis of the moths' paths during feeding bouts. While the increase in energy out is statistically significant (ANOVA, $P < 0.05$), it is minute compared with the energy in. (C) Rate of energy gain. Energy gain patterns mimic patterns of feeding rate.

mechanosensory information from flowers has significant impacts on flower handling in *M. sexta*. Additionally, floral genes that have a strong effect on pollinator preference are known to drive adaptation in some plant–pollinator systems (Schemske and Bradshaw, 1999). It is reasonable to hypothesize that any floral trait impacting pollinator fitness could be subjected to selection. Although some essential amino acids found in Lepidopteran eggs can only be derived from larval diet (O'Brien et al., 2002), adult nectar meals contribute significantly to egg production (Hill, 1989). Because flower motion dynamics affect net energy gain, they could logically impact hawkmoth fitness. Given that, the biomechanical properties of hawkmoth-pollinated flowers could potentially be under selection.

However, this would require that hawkmoths actively prefer to feed from flowers with the motion dynamics that have the highest fitness benefit (i.e. those flowers which they gain the most energy from). In addition, the specificity of plant–pollinator interactions may be important. The data presented here suggest there is a clear need for further studies of flower-tracking in hawkmoths, a unique and exciting system in which to examine intricate plant–pollinator relationships.

Appendix

University of Washington Manduca sexta diet ingredients

Distilled water, 2230 ml; gelcarin, 35 g; wheat germ, 240 g; casein, 108 g; sucrose, 96 g; Wessons salt, 36 g; Torula yeast, 48 g; cholesterol, 10.5 g; sorbic acid, 6 g; methyl paraben, 3 g; ascorbic, 15 g; streptomycin, 0.6 g; kanamycin, 0.16 g; 10% formalin, 70 ml; raw linseed oil, 12 ml; nicotinic acid, 30 mg; riboflavin, 15 mg; thiamine, 7 mg; pyridoxine, 7 mg; folic acid, 7 mg; biotin, 0.6 mg.

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