Supplemental Information

Supplemental Figures

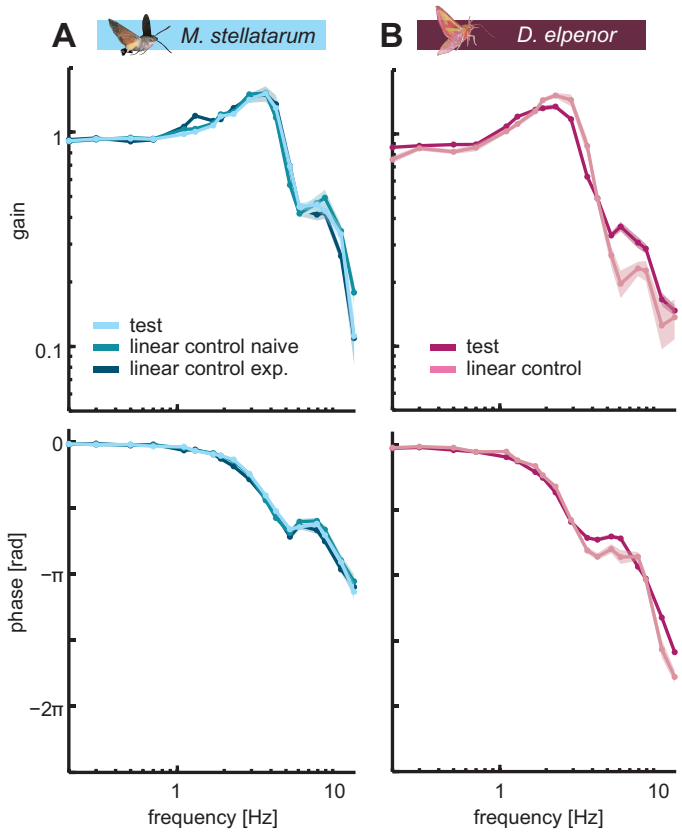


Fig. S1 **Linearity and experience tests of flower tracking**. Linear dynamics are characterized by scaling and superposition of response. To test the linearity of the experimental trials in *M.stellatarum* (A) and *D.elpenor* (B), linear control trials halved the amplitude of the flower's movement to test scaling and the phase of each constituent sinusoid were randomized to test superposition [1,2]. *M sexta* linearity was established in prior work using similar experiments [2]. Individuals of *M.stellatarum* were additionally tested for learning effects: naïve animals were those tracking for the first time, while experienced ("exp.") animals had completed a full flower tracking trial at least once.

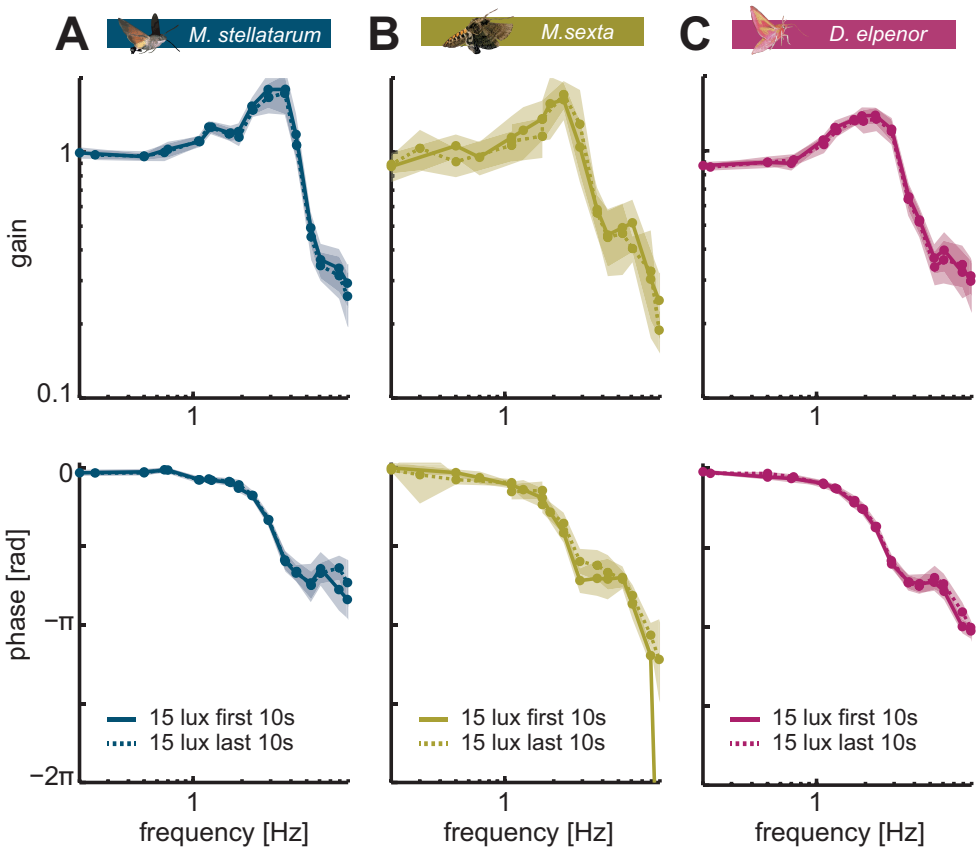


Fig. S2 **Time invariance test of flower tracking**. We compared the first 10s of tracking (solid lines) with the last 10s of tracking (dashed lines) for all three species and light conditions (*M.stellatarum* (A), *D.elpenor* (B) and *M sexta* (C), shown is data collected at 15 lux). There was no difference in tracking between these two intervals, suggesting that the tracking responses in our behavioural paradigm were time invariant, at least over the timecourse of the experiment.

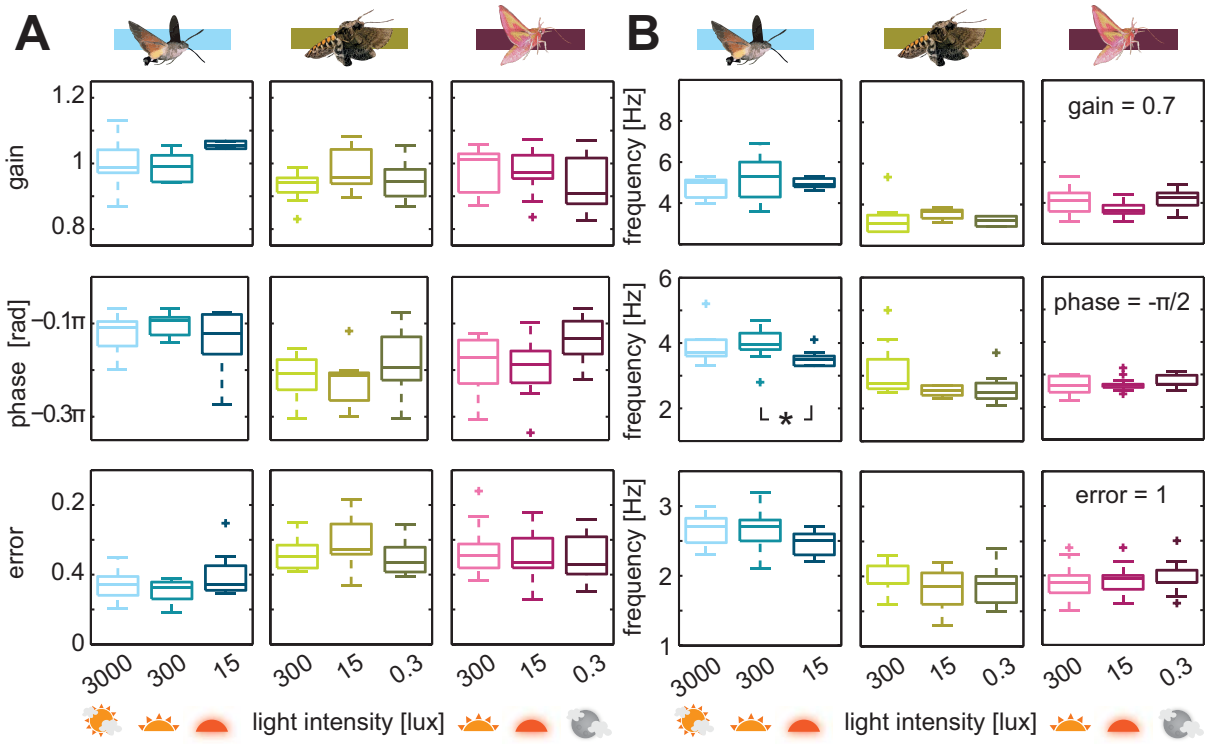
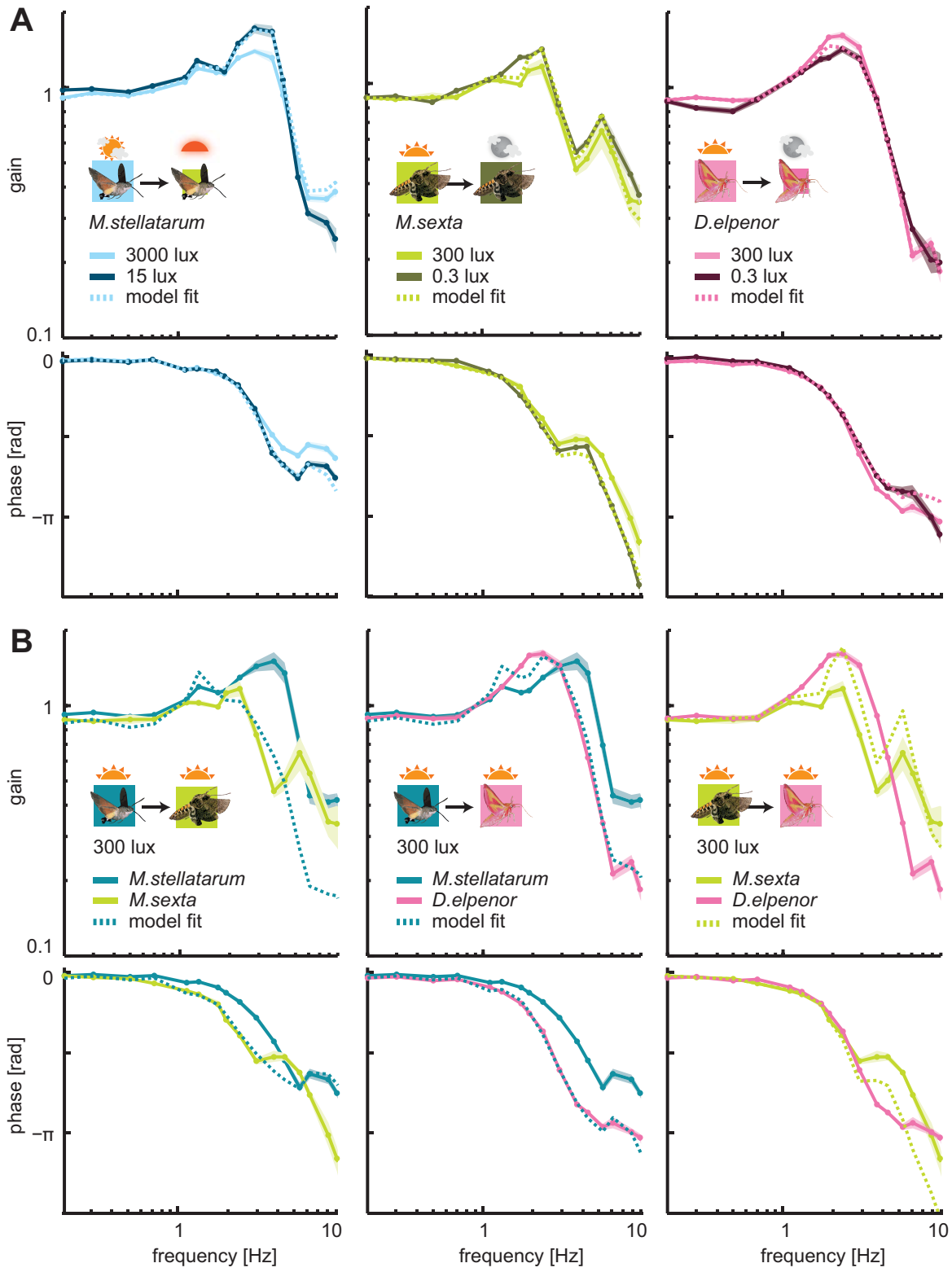


Fig. S3 **Summary statistics of flower tracking within species**.

A) Average gain, phase lag and tracking error of the diurnal (*M.stellatarum,* blue), crepuscular (*M.sexta,* green) and nocturnal (*D.elpenor,* red) species were calculated for the low frequency band (0.2-1.3 Hz).

B) Shown are the frequencies where each species' flower tracking reached 50% of its power (a gain of 0.7 or -3 dB), a phase of -π/2 and a tracking error of 1.

Box shows interquartile range and median, whiskers denote quartile ± 1.5 × interquartile range. Asterisks denote statistically significant differences ($p<0.05$, Kruskall-Wallis test).


A
gain
1
0.1
phase [rad]
0
−π
M.stellatarum
3000 lux
15 lux
model fit
M.sexta
300 lux
0.3 lux
model fit
D.elpenor
300 lux
0.3 lux
model fit
B
gain
1
0.1
phase [rad]
0
−π
300 lux
M.stellatarum
M.sexta
model fit
300 lux
M.stellatarum
D.elpenor
model fit
300 lux
M.sexta
D.elpenor
model fit
1
10
frequency [Hz]
1
10
frequency [Hz]
1
10
frequency [Hz]

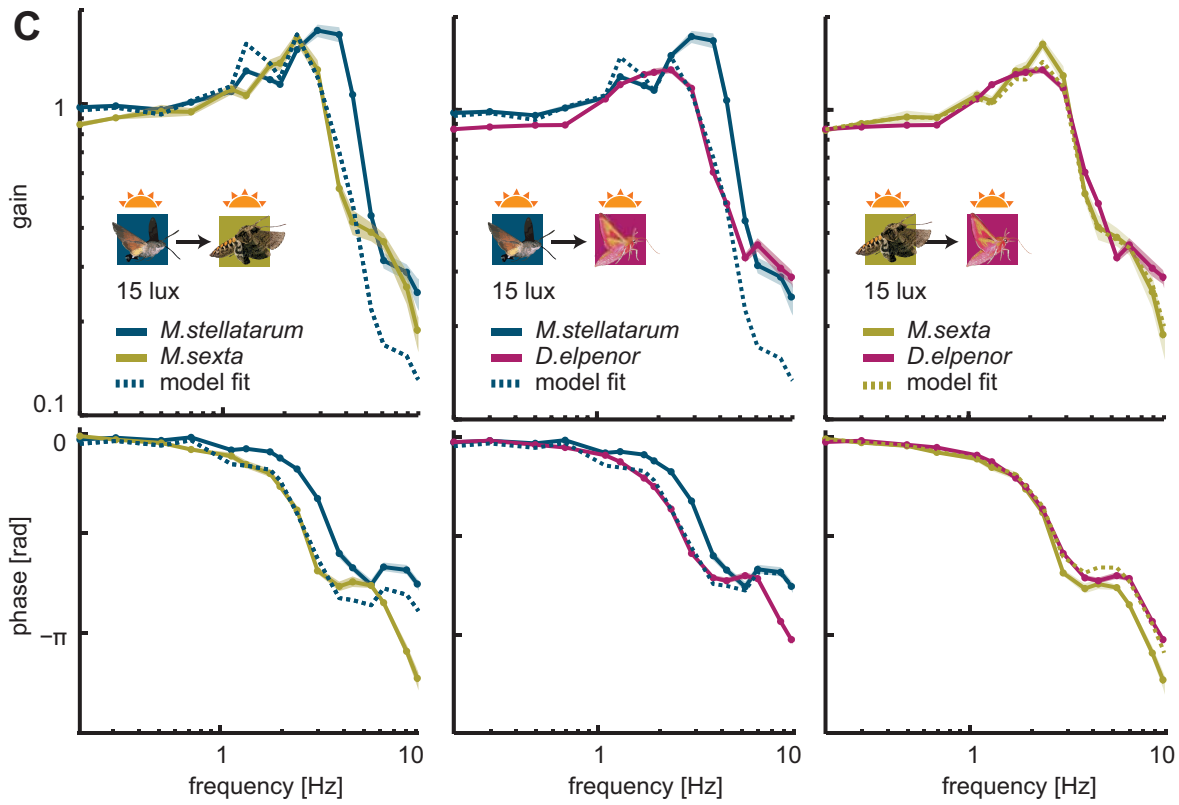


Fig.S4 **Model fitting of interspecific differences in flower tracking**. A) Simple delay fits without scale factors within species, fitted from the highest to the lowest tested light intensity (intensities and species indicated in panels). B) Model fits with delay and scale factor across species at 300 lux and 15 lux(C). Species fitted are indicated in the panels. See Table 1 for model results.

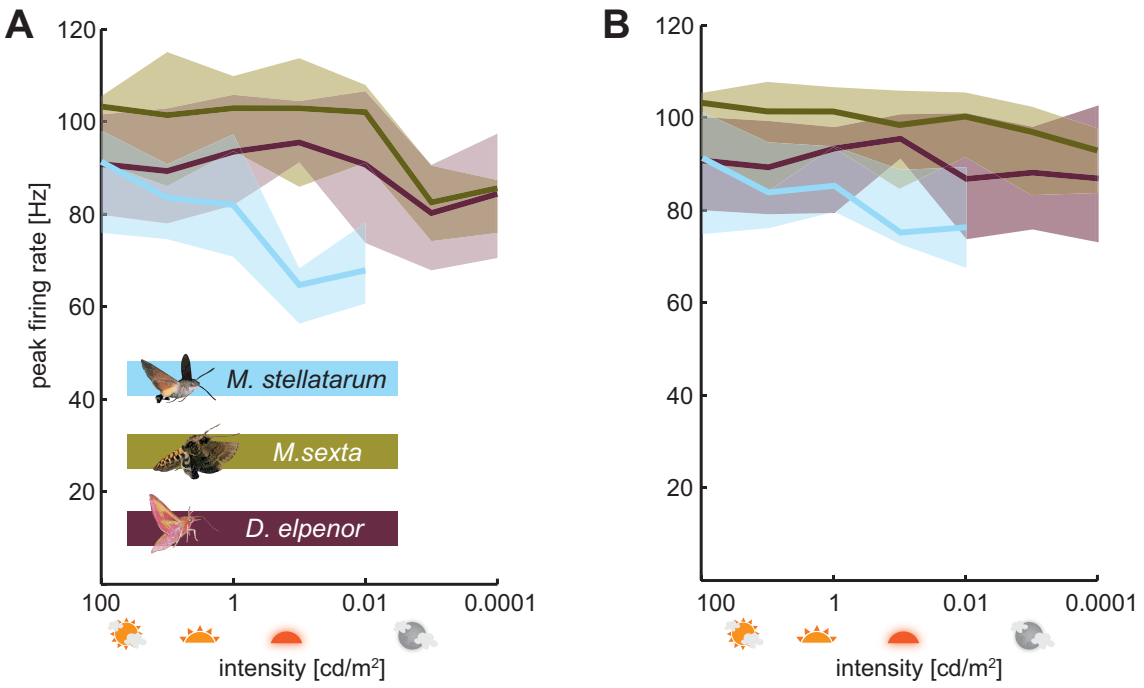


Fig.S5 **Peak firing rate of wide-field motion sensitive neurons in the three hawkmoth species**. The firing rate was determined from responses to moving sinusoidal gratings of different spatial and temporal frequencies at 25% (**A**) and 50% contrast (**B**) and re-plotted from [3]. Solid lines are median values, and shaded areas represent the inter-quartile range. The symbols on the intensity axis represent comparable light conditions in our behavioural experiments.

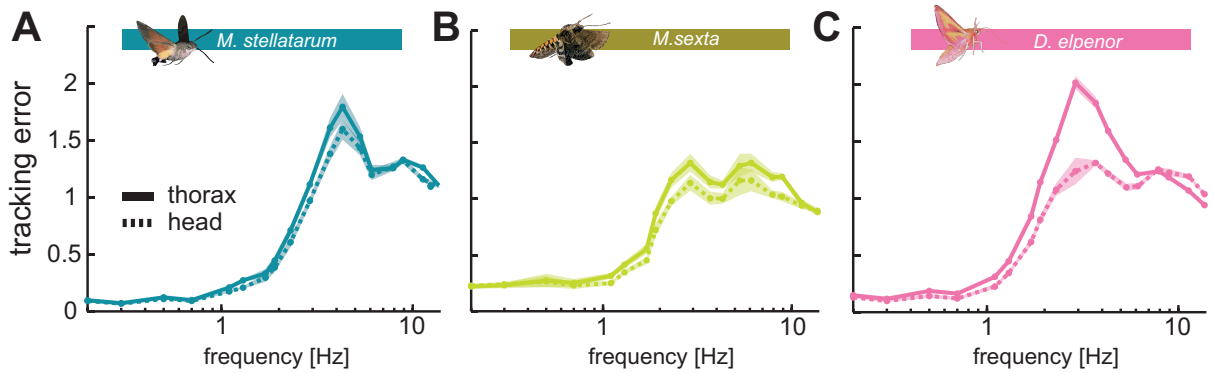


Fig.S6 **Head tracking of flower movement**. Tracking error of the head and thorax of the diurnal (A), crepuscular (B) and nocturnal (C) species at 300 lux. Tracking error of the head and thorax was not significantly different in the diurnal species, only significantly different at high frequencies in the crepuscular species, and significantly different at most frequencies in the nocturnal species (two-factor ANOVA, with tracking position as between and frequency as within group factor, *M.stellatarum* (all frequencies): tracking position: F=.27, p=0.61, frequency: F=0.24,p=0.99, *M.sexta* (all frequencies): tracking position: F=1.08, p=0.30, frequency: F=0.43,p=0.97, *M.sexta* (1.7-11.3 Hz): tracking position: F=4.6, p=0.04, frequency: F=0.96,p=0.73, *D.elpenor* (all frequencies): tracking position: F=5.26, p=0.02, frequency: F=0.11,p=1).

Supplemental Tables

Table S1 Model fits within species including a scale factor do not give substantially better fits than with only a delay (Table 1). Fits across species with only a temporal delay and no scale factor generally do not fit as well as the model that includes both (Table 1).

			delay [ms]	scale factor	% explained	SSE
within	highest lowest	*M.stellatarum*	-8.5	0.96	84	0.13
		M.sexta	-10	0.98	79	0.15
		D.elpenor	6	1	72	0.1
across species, no scale factor	300 lux	*M.stell. - M.sexta*	14.7		10	6.9
		M.stell. - D.elpenor	20.7		31	3.3
		M.sexta - D.elpenor	-17.4		35	1.7
	15 lux	*M.stell. - M.sexta*	16.2		28	3.3
		M.stell. - D.elpenor	20.6		25	5.1
		M.sexta - D.elpenor	7.4		66	0.14
	0.3 lux	*M.sexta - D.elpenor*	0		0	1.5

Supplementary Videos

Video S1

High-speed overhead video (slowed to 25% speed) of the diurnal *Macroglossum stellatarum* tracking a robotic flower. The tracked position of the thorax (orange) and flower (white) are outlined.

Video S2

High-speed overhead video (slowed to 25% speed) of the crepuscular *Manduca sexta* tracking a robotic flower. The tracked position of the thorax (orange) and flower (white) are outlined.

Video S3

High-speed overhead video (slowed to 25% speed) of the nocturnal *Deilephila elpenor* tracking a robotic flower. The tracked position of the thorax (orange) and flower (white) are outlined.

Supplemental Methods

Animals

In this study, we investigated three species of hawkmoths (Lepidoptera: Sphingidae): the nocturnal elephant hawkmoth *Deilephila elpenor*, the crepuscular tobacco hornworm moth *Manduca sexta*, and the diurnal hummingbird hawkmoth *Macroglossum stellatarum.*

M. stellatarum were cultured from wild-caught individuals collected in France (Sorede). *D. elpenor* pupae were purchased from a commercial supplier (Neil West, Newark, UK) and were kept at 4°C until use. They were stimulated to eclose by transferring them to 26°. Moths of both species were held on a 14:10 day:night light regime in flight cages at Lund University and fed with sugar solution for some days before starting experiments. Data for *M. sexta* are taken from the same data sets as previously published [2]. In brief, moths were reared at the University of Washington (Seattle, WA, USA) colony on a 12:12 day:night regime. Individuals were tested under dark-adapted conditions, 2 to 5 days post-exclosion without prior exposure to the artificial flowers.

Behavioural Experiments

Light intensity measurements

In order to ensure comparable light levels between experiments, we measured the incident light immediately in front, and immediately to the left and right of the flower, before each experiment. The light meter was directly oriented towards the light source, and thus we obtained a measure of illuminance (lux). We also measured the luminance of the flower face 2 cm in front of the flower, as an average of four values above and below, as well as light and right of the nectary (see Fig. 1A for illuminance and luminance values in the different experimental conditions). Since the luminance values reported in physiological studies of hawkmoth visual systems are screen averages of both brighter and darker stimulus parts, while our flower face is likely the brightest part of the moth's visual field, we measured the luminance of the background directly adjacent to the flower (4 times dimmer on average than the flower face), to construct an average luminance measure. Using this measure, our behavioural light intensities 3000, 300, 15 and 0.3 lux would correspond to 46, 4, 0.2, 0.003 cd/m^2. *Differences between experimental conditions*

Since the previous *M.sexta* study only provided us with data at 300 and 0.3 lux [2], we recorded data from *M.sexta* at 15 lux at the Georgia Institute of Technology. The trials match the experimental protocol for *M. stellatarum* and *D. elpenor* trials, but used an updated motor system and in some cases different camera views compared to the 300 and 0.3 lux data from [2]. *M. sexta* 15 lux data did diverge from the 300 lux and 0.3 trials at the higher frequencies and these differences may arise from differences in set-up. However, they do not affect the conclusions of this study. Intraspecific comparisons were from the brightest to dimmest conditions and consistent across species. Interspecies fits showed poor fits of the diurnal or nocturnal species to M. sexta regardless of luminance (Table1, S1).

There were a number of small differences between experimental conditions for *M.sexta* compared to *M.stellatarum* and *D.elpenor*. The following arose from a need to maximise experimental outcome per animal on the latter two species, as their abundance was limited: while all *M.sexta*

moths were naïve moths, which had not fed from the artificial flowers prior to experiments, *M.stellatarum* and *D.elpenor* were exposed to the artificial flowers prior to experimentation, by letting them feed from stationary flowers in their holding cages ad libitum in the days before experiments started. To induce foraging animals were starved for 12 h before experiments (and at least 36h for the 300 lux condition in *D.*elpenor). To encourage flower identification, we placed a small blue ring made of cardboard around the nectary, as both species have a natural preference for blue in flower choice, and thus were more attracted to a flower containing blue. We left the majority of the flower face white, in order to have comparable illuminance conditions to the experiments on *M.sexta.* To test if the effect of experienced moths would affect tracking compared to Naïve animals, we compared naïve and experience M. stellatarum moths and found no significant differences in tracking (Fig. S1). This comparison was not possible for *D. elpenor* given the limit supply of wild-caught animals. A 20% sugar solution, scented with commercially available bergamot essential oil (Naissance, UK), instead of a seven component mixture mimicking the scent of *Datura wrightii* as in the *M.sexta* experiments, was used to fill the artificial flowers. Animals were adapted to the ambient light intensity in the arena prior to flower tracking (for at least 30 min), instead of dark adapting them before experiments.

System Identification and Data Analysis

Flower tracking can be described by two components: the gain and phase [2,4]). The gain describes the amount by which the moth moves at each frequency as a multiple of the amplitude of flower movement. Thus, a gain of 1 means identical tracking of the flower amplitude, a gain lower than 1 means moths move less than the flower, and a gain higher than 1 means they overshoot the flower movement (Fig.2, first row). The phase component describes the degree to which the moth is in synchrony with the flower movement as the difference between the flower's and the moth's position in the period of each sinusoidal stimulus component. At a phase of 0 the moth is in perfect synchrony with the flower and a negative phase means the moth is lagging behind the flower, (Fig.2, second row).

Gain and phase of the digitized tracking responses were calculated as the magnitude and angle of the complex valued response of the moth to the flower. Since phase is a circular value, values of π are equivalent to - π. If subsequent frequencies had a phase shift greater than π/2, we assumed that the higher frequency has a greater phase lag, and thus unwrapped the phase between these two frequencies as in [6]. Doing so gave easier visualization across individuals, but did not affect our modelling results because the fitting and averaging were performed in the complex plane.

Tracking error [2,5] was calculated as a metric to assess how well the moth tracked the flower. A gain of 1 and a phase lag of 0 would mean perfect tracking, while any deviation either in gain or phase would reduce tracking fidelity. We used the complex distance between the moth's response *H(s)* and the ideal tracking conditions (gain=1, phase lag=0) to define the tracking error, ε, as:

$$\varepsilon(s) = \|H(s) - (1 + 0i)\| \quad (1)$$

Here *s* is the Laplace frequency variable.

The frequency response of a dynamic system is often characterized by frequencies at which specific response values are achieved. For the gain, the corner or cut-off frequency of a dynamic response is the frequency at which the power in the tracking response falls below 0.5 (gain = 0.71). When phase lags further than $\pi/2$ radians then the system is more than a quarter period out of sync. For frequencies where the tracking error exceeds 1, the moth would do better to simply stay stationary (tracking with a gain of zero at the origin in the complex plane). However, tracking errors above 1 may occur because the moths use the same dynamics to track the whole range of frequencies in the stimulus. That is to say, the moth's dynamic response is a singular characteristic across all frequencies. . We therefore also compared the frequencies at which the tracking behaviour equalled characteristic gain, phase lag and tracking error values (gain = 0.71, phase = $-\pi/2$, tracking error = 1) using Kruskall-Wallis tests (across light conditions: Fig. S1, across species: Fig.4).

Within species, across light intensities, there was no significant difference in the frequencies at which characteristic gain, phase lag and tracking error values were reached (Fig. S1B). In order to analyse which aspects of tracking behaviour did change with light conditions, we split the responses in two frequency bands: the frequencies in which natural flower movements occur were used as a low frequency band (0.2-1.7Hz), while frequencies above, from 1.7 - 8.9 Hz where the high band. Above 8.9 Hz not all moths track the flower coherently [2] and the tracking error returns close to 1. We averaged gain, phase and tracking error in these two frequency bands for each light condition, and analysed the difference between conditions using Kruskall-Wallis tests (Fig.3, Fig. S1).

Fitting simple delays and scaling factors to the differences within and between species

In order to obtain possible explanations for the differences in flower tracking between light conditions and species, we used a closed-loop model described in [2] (Fig.1D). The sensory system registers the error between the moth's movement and the flower's movement, and sends this perception to the motor systems, which initiate a muscular response, acting on the flight mechanics of the moth. This will lead to a change in the moth's tracking position, which in turn generates (together with the flower movement) a new sensory error between flower and moth motion, which starts the closep-loop over once more. The response of the moth to any given frequency (denoted s – the Laplace variable), can be described by behavioural frequency response $H(s)$ that transforms the flower's movement in the moth's movement (Fig. 1D).

$H(s)$ is a complex-valued response whose magnitude is the gain of moth's movement with respect to the flower and the argument is the phase relationship. For each species of moth, flying at each given luminance condition there is a different behavioural response.

To determine if simple models of neural processing changes to difference luminance levels can account for the within and across species differences we model the differences between behavioural responses using two simple elements: 1) a simple delay, which is consistent the slowing of nervous processing, such as has been observed in the visual system of insects when light levels decrease 2) an open loop gain factor, which we termed scale factor a, which changes the strength of the responses to the perceived error between flower and moth motion, such as could resulting from changes in visual sensitivity due to spatial and temporal summation in dim light, a change in gain between sensory systems, or a chain in gain between sensory and motor systems.

Since the scale factor and delay capture neural processing they act on the inside of the closed loop. (Fig.1D) and must therefore be applied to the open loop response of the system *G(s)*. The open-loop response of a linear feedback system (with unity feedback gain) is related to the closed-loop response as:

$$G(s) = \frac{H(s)}{1 - H(s)} \tag{2}$$

In the frequency domain, a scale factor remains as a simple multiplicative gain term, and a time delay τ has the form of an exponential, $e^{-s\tau}$, where s is again the frequency variable. The predicted open-loop response for the lower luminance condition $G_d(s)$ is the scaled, delayed version of *G(s)*:

$$G_d(s) = a\, e^{-s\tau} \frac{H(s)}{1 - H(s)} \tag{3}$$

Putting this inner loop back into the closed loop results in the final delayed and scaled model of moth flower tracking $\hat{L}(s)$:

$$\hat{L}(s) = H_d(s) = \frac{G_d(s)}{1 + G_d(s)} = \frac{a\, e^{-s\tau} \left[\frac{H(s)}{1 - H(s)}\right]}{1 + a\, e^{-s\tau} \left[\frac{H(s)}{1 - H(s)}\right]} \tag{4}$$

In general, we used the complex average data of one experimental condition as the template *H(s)* to obtain $\hat{L}(s)$ from, and fitted $\hat{L}(s)$ to the complex average data of the condition we wanted to compare it to, *L(s)*. For within species comparisons, we used the highest luminance condition as the template and fitted it to the lowest luminance condition (therefore the terminology *H(s)* and *L(s)*). For between species comparisons we used the species naturally active in higher light conditions as the template. This was mainly a convention, since model results reversed when conditions were reversed. We initiated the model with a delay of 0ms, and a scale factor of 1 (equivalent no difference in open-loop gain).

Fitting was performed using an unconstrained nonlinear optimization algorithm (*fminsearch* in Matlab), by minimizing the sum of squared errors (*SSE*) between $\hat{L}(s)$and *L(s)* in the complex plane:

$$SSE = \sum_s \left\| \hat{L}(s) - L(s) \right\|^2 \tag{5}$$

A range of initial conditions (delay from 50ms to -50; scale factor from 5 to 0.1) were tested to ensure that local minima did not alter the results. To obtain a goodness-of-fit metric (*GoF*), we calculated the percent proportion of the squared distance between the complex averages of the two conditions which was accounted for by the model prediction (and thus obtained a metric similar to r^2, the proportion of variance explained):

$$GoF = \left(1 - \frac{SSE}{\sum_s \|H(s) - L(s)\|^2}\right) * 100 \quad (6)$$

We could not find consensus on a metric for goodness of fit in the complex plane. An alternative measure might be to calculate SSE in the log-complex rather than complex plane which weights differences at low gain more strongly. We repeated all fits using data transformed into the log complex plane yet these did not provide better fits than the complex SSEs, and we thus remained with the latter method.

Linearity and experience controls

The frequency response to a sum-of-sines stimulus is a captures the response of the moth to any arbitrary motion of the flower (within the frequency bounds) provided the system is linear. Linear systems have the property of scaling and superposition. We tested whether the responses of our moths were dependent on the specific stimulus amplitude (scaling) or the relative phases of each constituent sinusoid (superposition). To this aim, we created a stimulus with different amplitude (50% of the original amplitude) and randomised phases, as [2] did for *M.sexta*, and compared the tracking behaviour in this condition to the tracking obtained with the original stimulus. Stimulus conditions did not have a significant effect on tracking behaviour in either species (Fig. S1A, two-factor ANOVA, with frequency as within groups effect, and stimulus condition as between groups, tests for *M.sexta* in [2], *M.stellatarum*: gain: F=0.29, p=0.59, phase: F=0.22, p=0.64, tracking error: F=1.73, p=0.18, *D.elpenor*: gain: F=2.26, p=0.13, phase: F=0.42, p=0.51, tracking error: F=1.63, p=0.19). Furthermore, we did not find any evidence for time-dependent effects in the responses, when we compared the first 10s of tracking with the second section of 10s in any of the three species (Fig.S2).

Some of the individuals tracked in more than one condition. In order to rule out effects on flight performance caused by experience of flower tracking, we had 10 individuals of *M.stellatarum* perform flower tracking experiments twice on consecutive days, using the same stimulus characteristics (Fig.S1A). There was no significant difference between the trials of experienced and naïve moths with respect to gain, phase or tracking error (two-factor ANOVA, with experience level and frequency as within group effects, effect of experience level on gain: F=0, p=0.95, phase: F=0.63, p=0.42, tracking error: F=054, p=0.46), suggesting that experience does not influence flower tracking performance.

Head position while flower tracking

It is a reasonable assumption that the thorax best represents the flight control output of the moth (as this is where the wings insert and thus steering takes places). However, it is the proboscis, which extracts the nectar from the flower, and thus, in terms of feeding success, the head's tracking performance is essential. Interestingly, there was a significant difference in tracking error between the head and thorax tracking in the nocturnal species, a smaller differences in the crepuscular one (significant only at high frequencies) and no significant one in the diurnal species (Fig.S6). Thus, the nocturnal species (and to a lesser degree the crepuscular one) improved its tracking performance through compensatory head movements. Overall, the diurnal species still tracked significantly better even comparing head position (two-factor ANOVA, with species as between and frequency as within

group factor, species: $F=8.59$, $p<0.004$, frequency: $F=0.38$,$p=0.91$), suggesting that head movements alone cannot compensate for the superior tracking performance of the diurnal species.

References

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