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Male moths optimally balance take-off thoracic temperature and warm-up duration to reach a pheromone source quickly

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Abstract

Animal activities, such as foraging and reproduction, are constrained by decisions about how to allocate energy and time efficiently. Overall, male moths invest less in reproduction than females, but they are thought to engage in a scramble competition for access to females that advertise readiness to mate by releasing sexual pheromones. However, before male moths can follow the pheromone, they often need to heat their flight muscles by shivering to produce sufficient power for sustained flight. Here, we show that *Helicoverpa zea* males that sense the female pheromone at high ambient temperatures take off with higher thoracic temperature, shiver for less time and warm up faster than males tested at lower ambient temperatures. These higher take-off temperatures translate into higher airspeeds, underscoring the importance of thoracic temperature for flight performance. Furthermore, shorter combined duration for warm-up and pheromone-mediated optomotor anemotaxis is consistent with the idea that males engage in scramble competition for access to females in nature. Our results strongly suggest that male moths minimize the time between perceiving the female's pheromone signal and arriving at the source by optimizing thermoregulatory behaviour and temperature-dependent flight performance in accordance with ambient temperature conditions. Our finding that moths engage in a trade-off between rapid flight initiation and suboptimal flight performance suggests a sensorimotor control mechanism that involves a complex interaction with the thermal environment.

Keywords

heliathine; insect; olfaction; moth; physiology; scramble competition; shivering; thermobiology; thermoregulation; wind tunnel

Every organism must take into account the efficient allocation of energy and time as it engages in fundamental activities such as foraging and reproduction. The study of how organisms maximize their use of energy and time provides insight into their ability to adapt

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to a changing environment (Pianka, 1994). Animals search for appropriate sources of food/water, mates and oviposition sites for growth and reproduction (Bell, 1991). However, such searching behaviour has costs that animals must balance with the potential benefits gained from the resource. Costs include energy expended on movement itself, time taken away from other activities and risk of predation while searching (Bell, 1991). Furthermore, whether searching for food or mates, animals face the general problem of optimizing their searching strategies to outcompete conspecific competitors for the same resource. Optimal food foraging and food resource utilization have been shown repeatedly across taxa (e.g. Heineman, Springman, & Bull, 2008; Jensen et al., 2012; Mukherjee, Zelcer, & Kotler, 2009). However, optimization in mate-finding strategies has been difficult to quantify. Here we investigate factors that influence the optimization of mate localization in male moths.

The attraction of male moths to female pheromones is a well-established model for long-distance sexual communication (Mafrá-Neto & Cardé, 1994; Vetter & Baker, 1984; Vickers, Christensen, Mustaparta, & Baker, 1991; Willis & Arbas, 1991). During pheromone-mediated upwind flight, male moths are considered to be scrambling for females and, thus, bear the major costs of finding a mate (i.e. energy expenditure and risk; Greenfield, 1981; Thornhill & Alcock, 1983). Scramble competition occurs when a finite resource that is shared between competitors, such as a sexually receptive female, is reduced with increasing population density. This type of intraspecific competition selects for phenotypic traits such as enhanced sensitivity to sex pheromone detection (e.g. seen in sexually dimorphic antennae; Kaissling, 2009) and better searching and wind-tracking abilities (Vickers, Christensen, Baker, & Hildebrand, 2001; Wyatt, 2003), which favour early arrival at a calling female (Lloyd, 1979; Wyatt, 2003). Furthermore, female receptivity and sex pheromone production rapidly decrease after mating (Barth, 1968; Gillot & Friedel, 1977; Raina, Klun, & Stadelbacher, 1986; Webster & Cardé, 1984) as oviposition behaviours are initiated (Raina & Stadelbacher, 1990). Males arriving first are likely to mate with a female, and thus, early arrival is critical for fitness. Successful location of a calling female depends critically on flight performance. Because muscle efficiency is strongly temperature dependent, over a wide range of ambient temperatures, flight muscles of endothermic moths need to be heated before a moth can engage in upwind locomotion (Dorsett, 1962; Heinrich, 2007; Heinrich & Mommsen, 1985; Krogh & Zeuthen, 1941).

Pre-flight warm-up behaviour serves the role of setting the stage for subsequent behaviour and was therefore predicted to occur at maximal heating rates in order to minimize time and energy expenditure before taking flight (Kammer, 1981). However, as is the case for other locomotor functions (Pearson, 1993), multimodal sensory information modulates preflight warm-up behaviour and influences muscle temperature at take-off in stimulated males of the corn earworm *Helioverpa zea* (Crespo, Goller, & Vickers, 2012). Males sensing the complete pheromone blend take off at lower thoracic temperatures, shiver for less time and heat up faster than males exposed to unattractive blends or control odours. The main mechanism involved in the olfactory modulation of the heating rate was shown to be the differential activation of motor units during each muscle contraction cycle in both antagonistic flight muscles (Crespo, Vickers, & Goller, 2013). The lower thoracic temperatures at take-off also were correlated with low lift production during tethered flight

(Crespo et al., 2012). However, the extent to which lower preflight thoracic temperature affects flight performance remains unclear. In the current study, we explore how take-off thoracic temperature influences pheromone-mediated optomotor anemotaxis of males under different ambient temperatures in seminatural conditions. We show that, at different ambient temperatures, pheromone-stimulated male moths minimize their searching time for females by varying the duration of their warm-up and in-flight periods. We propose that this time optimization process supports a scramble competition scenario where males that arrive at a calling female first are more likely to copulate with her than males arriving later.

Methods

Insects

Colonies of *H. zea* have been maintained at the University of Utah since 1998. Larvae were reared in an environmental chamber at 23 °C and 80% relative humidity on a modified pinto-bean diet (Shorey & Hale, 1965) until pupation. Pupae were then sexed (according to Butt & Cantu, 1962) and males were placed into environmental chambers (Percival Scientific, Boone, IA, U.S.A.) at 25 °C and 60% relative humidity on a 14:10 h light:dark cycle until adult emergence. Every day males were aged and separated in plastic containers with access to a 9% sucrose solution. Males of 2–6 days of age were utilized in experiments carried out between the third and sixth hour of scotophase (i.e. 3–6 h after the dark phase of the photoperiod; Vetter & Baker, 1983, 1984).

On the day of experimentation, males were carefully introduced into 3 × 3 cm (W × H) cylindrical wire-screen cages and left to acclimatize in a wind tunnel room for at least 1 h. Individual males were then allowed to take flight from a rubber stand by inverting their cage on top of it. This release stand was positioned in the horizontal centre of the wind tunnel, 40 cm from the downwind end and 24 cm above the wind tunnel floor, to intersect the pheromone plume.

Wind Tunnel

The wind tunnel at the University of Utah has a working section of 2.5 × 1.14 × 1.14 m (L × H × W). The temperature of the wind tunnel room was set to allow for testing under three conditions (mean ± SD): Cold: 19.6±0.4 °C, 34.9±10.7% RH and 4.4±2.7 cm/s wind speed; Room: 22.0±0.3 °C, 27.0±6.2% RH and 46.7±4.7 cm/s wind speed; Warm: 26.8±0.1 °C, 28.9±12.8% RH and 43.7±4.4 cm/s wind speed. Illumination was provided by red and white incandescent light bulbs that were independently controlled by rheostats. The odour plume was vented to the exterior of the building at the downwind end of the wind tunnel via a large exhaust duct. An infrared video camera (FLIR systems ThermoCAM® S65HS) above the take-off platform and inside the wind tunnel was used to record temperature changes in freely behaving insects. Once each male moth took flight, its track was recorded from a top view with a monochrome video camera (Panasonic WV-BP330) on a computer (see Data Analyses).

Pheromone Components

The blend and ratio of chemical compounds utilized in these experiments were based upon previous wind tunnel behavioural data and the known constituents of female *H. zea* pheromone gland components (e.g. Vetter & Baker, 1984; Vickers et al., 1991). Concentrated solutions of *cis*-11-hexadecenal (Z11-16:Ald) and *cis*-9-hexadecenal (Z9-16:Ald; Sigma Chemical Company, MO, U.S.A.; maintained at -20 °C) were used to make volumetric dilutions in hexane until a concentration of 100 ng/μl was achieved for Z11-16:Ald and 10 ng/μl was achieved for Z9-16:Ald (Vickers et al., 1991). The purity of each solution was checked by capillary gas chromatography (Shimadzu GC 17A). The odour source consisted of 1000 ng of Z11-16:Ald (main pheromone component) and 50 ng of Z9-16:Ald (secondary pheromone component) loaded onto a circular filter paper disk (Whatman No. 4, 1 cm diameter; Vickers et al., 1991). Once the two compounds were admixed on the disk, the hexane solvent was allowed to evaporate in the fume hood so that it was not a part of the odour mixture tested. The odour disk was held by an alligator clip mounted on a wire post with a heavy rubber cork base and positioned at the upwind end of the wind tunnel (24.5 cm above the wind tunnel floor; source replaced every 60 min). The distance between the take-off cage and the odour source was 1.70 m, but only the last 1.4 m of this distance were covered by the video camera.

Behavioural Assays

Prior to releasing the male moths in the wind tunnel, video recording from both the IR and monochrome video cameras was started. The IR video camera recorded the preflight thermoregulatory response of males to the pheromone source (Crespo et al., 2012) and the monochrome video camera recorded each male's subsequent flight track (e.g. Vickers, 2006). Male moths were carefully positioned in a wire-screen cage downwind from the pheromone source. Individuals were tested up to four times (always allowing them to cool down to ambient temperature between trials). Males that did not respond or did not take off within 5 min of the beginning of the experiment were checked for their ability to fly. Those incapable of flight were excluded from the results and those capable of flight were scored as 'nonresponders'. Males that took off but did not cast in response to the pheromone were also recorded as 'nonresponders' (see Data Analyses). Because of loss of data or inconsistencies in a few flight tracks, the total number of individuals was slightly lower for flight variables and time to complete the track.

Data Analyses

Recorded preflight thermal responses were analysed by using ThermoCAM Researcher Professional 2.8 SR-1. Thoracic surface temperature was transformed into core thoracic temperature using calibration data described in Crespo et al. (2012). Differences in warm-up behaviour (i.e. thoracic temperature at take-off, duration of shivering and thoracic heating rate during shivering) were subjected to a two-sample *t* test, Welch's two-sample *t* test or Wilcoxon two-sample test with a false discovery rate (FDR) correction for multiple comparisons (Benjamini & Hochberg, 1995; see Supplementary Tables S1–S8).

Male flight tracks were recorded using Noldus Ethovision XT 8.0 (Noldus Information Technology, Leesburg, VA, U.S.A.) but were then analysed with MtrackJ (Meijering,

Dzyubachyk, & Smal, 2012) and TAS (modified from the track analysis system furnished by Dr T. C. Baker, Pennsylvania State University). Only males that flew upwind to within 10 cm of the pheromone source were used to compare flight track variables, which included airspeed, ground speed, course angle and track angle (see Charlton, Kanno, Collins, & Cardé (1993) for terminology and conventions used for analyses of flight tracks). Significant differences between treatments for these flight track variables were identified by either a two-sample *t* test or a Wilcoxon two-sample test with a false discovery rate (FDR) correction for multiple comparisons (Benjamini & Hochberg, 1995; see Supplementary Tables S9–S19).

Flight tracks were divided into six sequential segments: (1) flight: male takes flight; (2) cast: male flies near the take-off platform while attempting to 'lock-on' to the odour plume; (3) ¼ way: male flies more than one-fourth of the distance (42.5 cm) between the release platform and the pheromone source; (4) ½ way: male flies more than half of the distance (85 cm) between the release platform and the pheromone source; (5) ¾ way: male flies more than three-fourths of the distance (127.5 cm) between the release platform and the pheromone source; (6) source: male flies upwind until he is within 10 cm of the pheromone source (i.e. male completes the track; including males that contacted the odour source). The percentage of males performing each behaviour were calculated and subjected to a chi-square 2×2 test of independence with Yates' correction for continuity and false discovery rate (FDR) correction (Benjamini & Hochberg, 1995) for multiple comparisons (see Supplementary Tables S20–S31).

Optimization models for long distance pheromone-mediated optomotor anemotaxis were constructed by multiplying the observed wind tunnel flight time by 100 to calculate the predicted total amount of time (warm-up time + flight time $\times$ 100) required for a male moth to arrive at a calling female 135 ± 5 m away for the three ambient temperatures tested. Models best fitting the data for the different ambient temperatures were as follows: cold: $y = 86.8 \times x^2 - 4900 \times x + 69958$; room: $y = 46.1 \times x^2 - 2756 \times x + 41842$; warm: $y = 41.3 \times x^2 - 2850 \times x + 49764$.

All statistical analyses were performed using the R statistical package (R Development Core Team, 2013).

Ethical Note

Experiments were conducted in accordance with animal welfare guidelines.

Results

Ambient Temperature Affects Warm-up Behaviour

Male moths exposed to cold ambient temperature (19.6 ± 0.4 °C) took off at a thoracic temperature of approximately 28.5 °C irrespective of whether they subsequently flew to the odour source or not ($t_{49.8} = 0.52$, $P_{\text{corr}} = 0.603$; Fig. 1a, Supplementary Tables S1–S3). However, at the two other ambient temperatures (room: 22.0 ± 0.3 °C; warm: 26.8 ± 0.1 °C), thoracic temperature at take-off was significantly higher (approximately 1 °C) for individuals that failed to fly upwind to the source (room: $t_{71} = -2.09$, $P_{\text{corr}} = 0.045$; warm:

$t_{83} = -2.83$, $P_{\text{corr}} = 0.008$). Interestingly, even though males were able to successfully fly upwind with a take-off thoracic temperature of 28.5°C (see cold ambient temperature), when exposed to higher ambient temperature, moths warmed up to mean ($\pm$ SE) thoracic temperatures of $30.2 \pm 0.3^{\circ}\text{C}$ and $33.9 \pm 0.2^{\circ}\text{C}$ for room and warm ambient temperatures, respectively. This increase in take-off thoracic temperature for warm ambient temperature was achieved by a significantly higher mean heating rate during warm-up (completed tracks: cold_C–warm_C: $t_{68} = 7.39$, $P_{\text{corr}} = 1.253\text{e}^{-9}$; room_C–warm_C: $t_{83} = 7.18$, $P_{\text{corr}} = 1.253\text{e}^{-9}$; Fig. 1c, Supplementary Tables S6–S8), which, in turn, reduced the duration of preflight warm-up (cold_C–warm_C: $W = 1095$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 7.945\text{e}^{-10}$; room_C–warm_C: $W = 1539.5$, $N_1 = N_2 = 42$, $P_{\text{corr}} = 1.873\text{e}^{-8}$; Fig. 1b, Supplementary Tables S4–S5).

Pheromone-mediated Upwind Flight Is Affected by Ambient Temperature

The flight tracks of male moths exposed to warm ambient temperature ($26.8 \pm 0.1^{\circ}\text{C}$) differed from those of males tested in cold ambient temperature ($19.6 \pm 0.4^{\circ}\text{C}$; Fig. 2). The elevated thoracic temperature resulted in a significant increase in mean ($\pm$ SE) airspeed from 73.59 ± 1.61 cm/s to 83.79 ± 3.37 cm/s when comparing cold to warm ambient temperature (cold–warm: $V = 654$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.005$; Fig. 3a, Supplementary Tables S9–S10). Consequently, mean ($\pm$ SE) ground speed also increased from 40.28 ± 1.54 cm/s to 49.33 ± 3.29 cm/s (cold–warm: $V = 616$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.020$; Table 1, Supplementary Tables S13–S14). However, mean ($\pm$ SE) track and course angles were approximately 47° and 20° , respectively, for all ambient temperatures (track angle: cold–room: $t_{67} = 1.03$, $P_{\text{corr}} = 0.412$; cold–warm: $t_{67} = 0.83$, $P_{\text{corr}} = 0.412$; course angle: cold–room: $V = 446$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.527$; cold–warm: $V = 456$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.527$; Table 1, Supplementary Tables S15–S19). Greater male airspeed during upwind flight to the pheromone source significantly influenced the time that males spent flying in the plume: males spent significantly less time flying in the plume at warm ambient temperature than they did at cold ambient temperature (cold–room: $V = 355.5$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.115$; cold–warm: $V = 303$, $N_1 = 27$, $N_2 = 42$, $P_{\text{corr}} = 0.043$; Fig. 3b, Supplementary Tables S11–S12). Males took approximately 1 s less to locate the pheromone source successfully at warm ambient temperature than they did at cold ambient temperature (over a direct flight distance of 140 cm).

Upwind Progress in the Pheromone Plume for Different Ambient Temperatures

Figure 4 shows the performance of male moths in a sequential series of flight behaviours when exposed to the female pheromone. Moths tested at warm ($26.8 \pm 0.1^{\circ}\text{C}$) and room ($22.0 \pm 0.3^{\circ}\text{C}$) ambient temperatures did not differ significantly when flying upwind to the pheromone source (except for casting behaviour; see Supplementary Tables S20–S31 for calculated t tests). In these treatments, the same percentage of individuals flew to within 10 cm of the odour source (i.e. 54% and 47% for warm and room conditions, respectively; $\chi^2_1 = 0.72$, $P_{\text{corr}} = 0.396$). However, when males were tested in the cold ($19.6 \pm 0.4^{\circ}\text{C}$) condition, only 31% were able to locate the pheromone source (cold–room: $\chi^2_1 = 4.73$, $P_{\text{corr}} = 0.044$; cold–warm: $\chi^2_1 = 9.90$, $P_{\text{corr}} = 0.005$; Supplementary Tables S30–S31). Thus, low ambient temperatures significantly affected the flight behaviour of male moths.

Males Optimize Time to Pheromone Source

If time optimization dictates the thoracic temperature at which a male moth can take off when sensing the pheromone, the observed thoracic temperatures at take-off should agree with the predicted take-off thoracic temperatures for which the combined time for preflight warm-up and flight is minimal. Figure 5 shows the best-fit models for the relationship between core thoracic temperatures at take-off and total time invested in arriving at a pheromone source located 135 ± 5 m away for the three ambient temperatures tested (cold, room and warm). Both cold and warm data were best modelled by quadratic equations with minimum predicted times of arrival of 685 s and 570 s, respectively (Table 2). At these durations, the prediction is that male moths should take off at a thoracic temperature of 28.2°C for the cold treatment and 34.5°C for the warm treatment. Both predicted take-off temperatures agree with the mean observed take-off temperatures in the wind tunnel (Table 2). The same was true for the room ambient temperature data. Although a cubic equation explained slightly more of the variance in the room ambient temperature data ($R^2 = 0.419$), we used a quadratic equation to be consistent with the other two treatments. According to this model for the room treatment, the fastest males take 628 s to arrive at the odour source and take off at a thoracic temperature of 29.9°C (Table 2). Again, this predicted take-off thoracic temperature agrees with the mean observed take-off temperatures in the wind tunnel.

Discussion

These results show that higher take-off thoracic temperature allowed for faster airspeeds during pheromone-mediated optomotor anemotaxis in *H. zea* males. At elevated ambient temperature, preflight heating rates and take-off thoracic temperature of male moths sensing the pheromone were higher than those of males tested at lower ambient temperature. These preflight thermoregulatory differences translate into flight performance differences. Males orienting to the wind in response to female pheromone at high ambient temperature flew significantly faster than those flying at low ambient temperature, without showing changes in course and track angles. Both the preflight warm-up and flight performance data indicate that male moths optimize time spent successfully locating and arriving at the pheromone source. Predictions of total time spent in warm-up behaviours and pheromone-mediated upwind flight over tens of metres support a model in which males scramble for females. Furthermore, these results also show that a higher percentage of males taking off at high thoracic temperature, and thus flying at higher speeds, successfully follow the pheromone plume and arrive at its source.

Preflight thermoregulation is necessary for endothermic insects to initiate and sustain flight (Heinrich, 1974). Temperature of flight muscles is increased by shivering before take-off to allow these muscles to function efficiently during flight (Heinrich & Bartholomew, 1971), therefore facilitating demanding behaviours, such as navigating an odour plume under natural conditions. In moths, as in all other animals, motor function is modulated by sensory information in order to generate and control movement (Pearson, 1993). Perception of female pheromone indicates a mating opportunity and causes modulation of preflight warm-up in *H. zea* males by increasing the thoracic heating rate, decreasing the duration of

shivering and lowering the take-off thoracic temperature (Crespo et al., 2012). Here we show that the preflight thermoregulatory adjustments of male moths influence their odour-mediated flight performance and ultimately their success in arriving at the location of the pheromone source. Interestingly, at high ambient temperature, not only did males heat their thorax faster, but they also took off with higher thoracic temperatures than males tested at low ambient temperatures (6 °C mean difference with cold treatment). Because males are capable of successfully locating the pheromone source when taking off at mean thoracic temperatures of around 28 °C (as shown in the cold treatment), the additional time spent shivering to reach a higher thoracic temperature at high ambient temperature has likely constituted a selective advantage. Crespo et al. (2012) showed that maximal lift during tethered flight is achieved by males heating their thorax to around 35 °C. Thus, high thoracic temperatures at take-off allow increased flight power output and, consequently, higher airspeeds when orienting with respect to an odour plume.

Flight manoeuvres used by male moths when navigating a pheromone plume are affected by both ambient temperature (Charlton et al., 1993) and wind speed (Mafra-Neto & Cardé, 1998; Willis & Cardé, 1990). Our results concur to some extent with those of previous studies. *Helicoverpa zea* males increased their airspeed and ground speed, as reported for the gypsy moth, *Lymantria dispar*, and the almond moth, *Cadra cautella*, but did not vary their track and course angles when flying towards an attractive odour at high ambient temperature. We propose that this significant increase in airspeed, as well as the higher percentage of males arriving at the pheromone source, observed at high ambient temperatures is the result of high take-off thoracic temperatures. Note, however, that pheromone-activated males that did not fly to within 10 cm of the source took off at higher thoracic temperatures than individuals that arrived within 10 cm of the odour source, at least in the room and warm ambient temperature treatments. A similar result was reported by Crespo et al. (2012) when *H. zea* males were exposed to incomplete pheromone blends or control odours.

In the present experiment, warm-up duration was significantly less at high ambient temperatures but still allowed males to achieve higher thoracic temperatures and fly faster. This translated into males spending significantly less time in flight before arriving at the pheromone source than at lower ambient temperature. Results reported here agree with the hypothesis that, in order to arrive quickly at a calling female, males must optimize time spent in preflight warm-up with temperature-dependent flight time. Scramble competition for females is likely to occur over a longer distance than that used in our wind tunnel. Unfortunately, data on the success of male moths sensing the pheromone over long distances in natural conditions are very difficult to collect, particularly for nocturnal species like *H. zea*. Elkinton, Schal, Ono, and Cardé (1987) reported the proportion of male *L. dispar* L. leaving take-off cages in the presence of a female pheromone at various distances in a forest. Although few males departing 120 m from the odour source (the maximum distance tested) were recaptured at the source location, this study showed that males are capable of upwind flight over tens of metres in their natural environment (David & Birch, 1989; David, Kennedy, & Ludlow, 1983). Thus, we calculated the predicted total amount of time required for a male moth to arrive at a calling female 135±5 m away for the three ambient

temperatures tested. The correlation between time spent locating the odour source and take-off thoracic temperature revealed that males take off at thoracic temperatures that optimize time expenditure when sensing the female pheromone.

Sexual selection theory predicts that the sex investing less in reproduction (usually males) will compete with each other for access to the other sex (Trivers, 2006). Male moths, as in the case for many other insect species, are considered to be in a scramble competition for females (Wyatt, 2003), and thus, in a race against time to outcompete conspecific rivals for access to females. In this study we report data supporting a scramble competition scenario, in which pheromone-stimulated males optimize the time spent during preflight warm-up and subsequent upwind flight under different ambient temperatures. We propose that preflight thermoregulatory changes observed in male moths generate a trade-off between suboptimal flight performance and rapid onset of directed flight as an important aspect of a ubiquitous male reproductive strategy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- At high ambient temperatures, male moths take off with higher thoracic temperature.
- Males also shiver for less time and warm up faster than at low ambient temperature.
- Higher thoracic temperatures at take-off allow males to attain faster airspeeds.
- Males minimize flight time by optimizing thermoregulatory behaviour.
- Males engage in scramble competition for access to females.

Figure 1.

Preflight warm-up behaviour of *Helicoverpa zea* males exposed to different ambient temperatures: (a) core thoracic temperature at take-off; (b) duration of warm-up; (c) thoracic heating rate during warm-up. Ambient temperatures: cold (19.6 °C); room (22.0 °C); warm (26.8 °C); subscript letters in the X-axis labels indicate whether males flew to within 10 cm of the source (C: complete track) or did not fly to within 10 cm of the source (I: incomplete track) at each ambient temperature. Sample sizes are indicated in parentheses. Significant differences ($P < 0.05$) between treatments connected with a horizontal square bracket are indicated by an asterisk above the corresponding bracket. Box plots show first quartile, median, third quartile and outliers (data points exceeding 1.5 times the interquartile range). Means are indicated by crosses inside the boxes.

Figure 2.

Representative flight tracks of *H. zea* males flying at cold (A), room (B) and warm (C) ambient temperatures. Flight tracks were recorded from above and progress from the bottom of page to the top. Filled circles on the flight tracks represent the position of the moth every 0.033 s. Black arrows show wind direction. Position of the pheromone source is indicated by the target. Scale bar = 10 cm.

Figure 3.

Flight track parameters of males arriving within 10 cm of the pheromone source at cold, room and warm ambient temperatures. (a) Airspeed during pheromone-mediated upwind flight (see also ground speed, Table 1). (b) Duration of pheromone-mediated upwind flight duration. Sample sizes are indicated in parentheses. Significant differences ($P < 0.05$) between treatments connected with a horizontal square bracket are indicated by an asterisk above the corresponding bracket. Box plots show first quartile, median, third quartile and outliers (data points exceeding 1.5 times the interquartile range). Means are indicated by crosses inside the boxes.

Figure 4.

Sequential flight behaviours of male moths in response to female pheromone at cold, room and warm ambient temperatures. We recorded each male's percentage performance in a sequential series of flight behaviours: male took flight (flight); male oriented to the pheromone plume (cast); male sustained upwind flight to within 127.5 cm (1/4 way), 85 cm (1/2 way), 42.5 cm (3/4 way) or 10 cm of the odour source (source). Different lower-case letters above symbols within each behavioural category indicate a statistical difference according to a chi-square test.

Figure 5.

Prediction of total time required for a male moth to arrive at a female advertising sexual readiness 135 ± 5 m away with respect to the male's thoracic temperature at take-off at cold, room and warm ambient temperatures. Total time was calculated by multiplying flight duration in the wind tunnel times 100 and then adding it to the observed warm-up duration. Table 2 shows other model parameters and predicted values.

Table 1
Mean $\pm$ SE additional flight track parameters of male moths flying under three ambient temperatures

Track parameter	Mean temperature (°C)			<i>P</i>
	19.6	22.0	26.8	
Ground speed (cm/s)	40.28 $\pm$ 1.54	46.14 $\pm$ 1.94*	49.33 $\pm$ 3.29*	< 0.02
Track angle (°)	49.87 $\pm$ 3.05	45.70 $\pm$ 2.58	46.58 $\pm$ 2.51	> 0.4
Course angle (°)	20.43 $\pm$ 1.60	20.19 $\pm$ 1.41	21.06 $\pm$ 1.46	> 0.5

* Significant difference when compared to the first treatment (19.6 °C; see Supplementary Tables S13–S19).

Table 2

Model predictions for male moths optimizing warm-up and flight times when sensing the female pheromone 135 ± 5 m away for the three ambient temperatures (cold: $y = 86.8 \times x^2 - 4900 \times x + 69958$; room: $y = 46.1 \times x^2 - 2756 \times x + 41842$; warm: $y = 41.3 \times x^2 - 2850 \times x + 49764$)

Treatment	R^2	Optimum predicted temperature (°C)	Observed mean temperature (°C)	Minimum predicted time (s)
Cold	0.244	28.2	28.6	685
Room	0.285	29.9	30.2	628
Warm	0.313	34.5	33.9	570