Animal Behaviour 98 (2014) 79–85

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

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

José G. Crespo*, Neil J. Vickers, Franz Goller

Department of Biology, University of Utah, Salt Lake City, UT, U.S.A.

ARTICLE INFO

Article history:
Received 16 June 2014
Initial acceptance 31 July 2014
Final acceptance 4 September 2014
Available online 25 October 2014
MS. number: A14-00500R2

Keywords:
heliothine
insect
moth
olfaction
physiology
scramble competition
shivering
thermobiology
thermoregulation
wind tunnel

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.

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 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 (Mafra-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

* Correspondence: J. G. Crespo, Department of Biology, University of Utah, Salt Lake City, UT 84112, U.S.A.
E-mail address: jose.crespo@utah.edu (J. G. Crespo).

http://dx.doi.org/10.1016/j.anbehav.2014.09.031
0003-3472/

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).

Preflight 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 *Helicoverpa 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 44.5 ± 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 ThermaCAM® 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 ThermaCAM Researcher Professional 2.8 SR-1. Thoracic surface