THERMAL AVOIDANCE DURING FLIGHT IN THE LOCUST *LOCUSTA MIGRATORIA*

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Summary

In this paper, thermal avoidance in tethered flying locusts is described for the first time. Changes in body posture examined using high-speed cinematography revealed that the animals responded to a laterally positioned heat source with contralaterally directed abdomen and hindleg ruddering, behavioural patterns resembling manoeuvres observed in collision avoidance and in response to auditory signals. The analysis also showed that, during stimulation, left and right forewing depression became asymmetrical during the downstroke but remained symmetrical during the upstroke. Hindwing depression and elevation remained symmetrical during stimulus presentations. Electromyographic recordings from the left and right first basalar muscles (M97; forewing depressors) showed that contralateral depressor muscle activity was advanced by 10–12 ms relative to that on the stimulated side. There was also an increase in burst duration on the contralaterally stimulated side and an increase in wingbeat frequency of approximately 3 Hz. Ablation experiments showed that removal of the antennal flagella, which are the site of previously described thermoreceptors, did not abolish thermal avoidance manoeuvres. We conclude that thermal avoidance is triggered by an infrared sensitivity that is not mediated by the compound eyes, the ocelli or the antennal flagella.

Key words: insect, *Locusta migratoria*, locomotion, steering, temperature, infrared sensitivity, motor pattern, flight, behaviour.

Introduction

Animals moving in a natural environment encounter a profusion of stimuli to which they must respond by changing course. These stimuli take many forms including abiotic features of the environment that could be hazardous and thus must be avoided, and biotic signals that attract or repel the animal (e.g. mating signals). Survival is dependent on proficient sensorimotor integration, and the neural mechanisms underlying such integration can be profitably investigated in the locust flight system.

Considerable information has accumulated about many different aspects of the neural mechanisms of the locust flight motor (for reviews, see Robertson, 1986, 1989, 1995; Kutsch, 1989; Orchard *et al.* 1993), and there is also a body of literature concerned with wing movements and motor patterns underlying the steering that maintains constant course direction during flight (for reviews, see Kammer, 1985; Rowell, 1988, 1993; Reichert and Rowell, 1986; Reichert, 1993). More recently, there has been interest in directed manoeuvres leading to changes in course during flight. It is clear that tethered locusts attempt to steer away from ultrasonic pulses resembling the echolocation calls of hunting bats (Robert, 1989; Robert and Rowell, 1992) and attempt to steer around visually detected obstacles in their immediate flight path (Robertson and Reye, 1992; Robertson and Johnson, 1993*a,b*). A possible stimulus for course changes during locust flight that has not yet been investigated is a radiant heat source.

Thermosensory input is an important source of information for all organisms. Predators, prey and mates may all be detected thermally. For example, the blood-sucking reduviid bug *Rhodnius prolixus* demonstrates short-range orientation in response to convective heat stimuli (Wigglesworth and Gillett, 1934*a,b*), and its relative *Triatoma infestans* can orient to an infrared source (Lazzari and Nunez, 1989). In particular, poikilothermic animals rely heavily on thermal signals to initiate behavioural strategies for thermoregulation. These include postural adjustments to maximize or minimize the surface area presented to a radiant heat source (akinetic behaviour) and locomotor adjustments which can be undirected (thermokinesis) or directed (thermotaxis). Thus, thermosensory input can provide signals that might trigger course changes in certain animals. Indeed, a species of wood-boring beetle (Buprestidae: *Melanophila acuminata*) travels long distances (95–160 km) to lay eggs in newly burned trees after forest fires by detecting the infrared radiation with sense organs located within pits in the coxal cavities of their mesothoracic legs (Evans, 1964).

Locusta migratoria is a species of locust native to semi-arid regions of equatorial Africa where ambient temperature and radiation from heated substrata can vary considerably, both temporally and spatially (Uvarov, 1977). Stationary locusts orient in response to radiant heat sources such as the sun, adjusting the body surface area presented depending upon

internal body temperature (Volkonsky, 1939, cited in Slifer, 1951). Moreover, when ambient temperature is spatially variable, locusts aggregate in specific regions to maintain thoracic temperatures in the behaviourally preferred range of 32–44 °C (Miles, 1985). Whereas preferred ambient temperatures result in akinetic behaviour, higher ambient temperatures increase both the generalized locomotor activity of locusts and their propensity to jump (Abrams and Pearson, 1982).

We are interested in identifying different environmental stimuli that generate intentional steering manoeuvres in flying locusts and our goal is to compare locomotor responses to different stimuli. Using abdominal deflections as indicators of steering direction, we tested whether tethered flying locusts generate steering manoeuvres in response to laterally located heat lamps with or without infrared transmission filters. We also monitored the most pronounced changes in the wing kinematics associated with presumptive steering manoeuvres in order to describe the pattern of behaviour more completely. Finally, we monitored associated changes in the pattern of activity of the first basalar muscles of the forewings as a preliminary investigation of the motor pattern underlying this behaviour.

The only thermoreceptors in locusts of which we are aware are located in coeloconic sensilla on the antennal flagella (Waldow, 1970; Altner et al. 1981). Each thermoreceptor has a theoretical resolving power capable of detecting a 0.6 °C change in the temperature of an air stream passing over the flagellum at $2.4 \, \mathrm{m \, s^{-1}}$ (Ameismeier and Loftus, 1988). Given this information, we also tested whether removal of both antennal flagella, and thus removal of about 2400 coeloconic thermoreceptors (Altner et al. 1981), would prevent orientation in response to a radiant heat source. In this paper, we describe thermal avoidance in tethered flying locusts and show that it is triggered by an infrared sensitivity that is not mediated by the compound eyes, the ocelli or the antennal flagella.

Materials and methods
Animals

Locusta migratoria L. of either sex were selected from a crowded colony maintained at 31 °C on a 16 h:8 h light:dark cycle at Queen's University. Only apparently healthy animals at least 2 weeks past imaginal ecdysis with intact wings were chosen.

Procedures for the thermal stimulation of flying locusts

Animals were dorsally tethered with wax by the pronotum to a rigid copper rod and suspended in front of a cylindrical wind-tunnel (14.5 cm diameter by 40 cm long) that produced a laminar flow of air at a rate of $3 \, \mathrm{m \, s^{-1}}$ (verified with a hot-wire anemometer). 250 W heat lamps (Chauffa 40, Globe Pak, Montreal, Quebec) were placed equidistant (7 cm) from and perpendicular to both sides of the animal. We monitored the timing of the thermal stimulus during all experiments using a Sensortek thermometer (model BAT-12) with a copper/constantan thermocouple placed close to the head of the animal (Fig. 1).

We used abdomen position as a measure of steering behaviour (Camhi, 1970) and monitored it in two ways. In one method, abdomen position was measured from video-taped trials. A Hitachi camcorder (model VM5200A) operating at $60 \, \mathrm{fields \, s^{-1}}$ with an electronic shutter speed of 1/1000 s was positioned behind the animal and recorded images onto high-quality VHS tape. In the other method, adapted from Sandeman (1968), changes in the capacitative coupling between a fine copper wire attached to the tip of the abdomen and a pair of laterally placed sensing electrodes indicated the direction of abdomen movement. We fastened the wire to the eighth (females) or ninth (males) subgenital plates on the abdomen with a small drop of wax and, in our opinion, this did not hinder the movement of the abdomen during our experiments. In some instances, the transducer signal was passed through a 10 Hz Krohn-Hite low-pass filter (model 3750) to eliminate high-frequency components from the waveform.

Thermocouple and abdomen transducer signals were displayed on a Hameg digital storage oscilloscope (model HM208) and either recorded onto VHS video tape using a
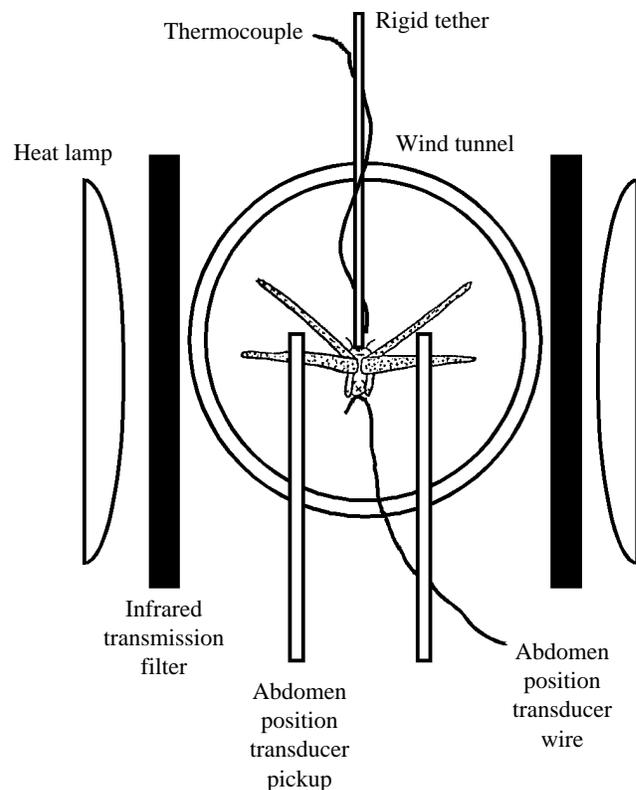

Fig. 1. Diagrammatic representation of the experimental arrangement used to record thermal avoidance in tethered flying locusts. Animals were tethered to a rigid copper rod in front of a cylindrical wind-tunnel between two heat lamps. Abdomen position was measured with an abdomen transducer (see Materials and methods) or from video-taped trials. For some experiments, infrared transmission filters (which only allowed long-wavelength infrared light to pass) were placed in front of the lamps to remove visible light from the stimulus. Temperature was monitored with a thermocouple positioned in front of the animal's head.

Neuro-corder (model DR-886) and Panasonic VCR (model HSU20) or recorded onto magnetic tape using a Vetter Instrumentation Recorder (model D). In either case, magnetic records were converted to hard copy using a Gould Electrostatic Recorder (model ES 1000) for analysis.

Recording of wing movements

We used a high-speed 16 mm Locam motion picture camera operating at 250 frames s^{-1} with a shutter speed of 1/1225 s to film three animals from behind during thermal presentations. All other aspects of the thermal presentation were as described above. We analyzed the films using the method described in Robertson and Reye (1992).

Tests for thermal avoidance steering

A control group of locusts (N=17) was flown without any thermal stimulus to provide a baseline of abdomen movements for subsequent comparison. The first group of test animals (N=14) was flown with the stimulus alternating between left and right sides. We switched the presentation side either instantly or leaving gaps of no stimulus lasting up to several minutes. The stimulus was usually presented for 20 s but ranged from 1 to 55 s. Inter-stimulus intervals were usually 10 s but ranged from 2 to 120 s. Since the heat lamps produce both visible and infrared radiation, we tested the second group (N=14) with infrared transmission filters (Edmund Scientific, Barrington, NJ, USA) placed in front of the heat lamps. These filters only allowed long-wavelength infrared light to pass. The last group (N=4) was tested with their eyes and ocelli covered with opaque nail-polish.

A separate group of locusts (N=7) was tested first with the heat lamps then with a 12 mm thick transparent acrylic barrier placed in front of the lamps. This allowed visible light to reach the animal but attenuated the infrared components of the stimulus.

We analyzed the abdominal responses recorded on video tape or using the abdomen transducer with a method modified from Doolan and Pollack (1985). Abdomen position was scored at stimulus onset and then 6 s into the presentation. When stimulus duration was less than 6 s (the situation for fewer than five animals of the total of 49), the score was taken at the end of the stimulus. A value of -1 was recorded for presentations in which the abdomen was closer to the stimulated side at the end of the trial relative to its position at the start of the trial; a value of 0 was assigned if the abdomen position was the same and a value of +1 was assigned if the abdomen was farther from the stimulated side. The scored values for stimuli presented on each side were averaged for each animal, yielding a mean response score (MRS). Using this method, positive MRSs indicated abdominal ruddering away from the stimulus and negative MRSs indicated ruddering towards the stimulus. We recorded ten stimulus responses from each locust, five from left presentations and five from right presentations. For control animals to which no stimuli were presented, we obtained scores from 10 s sham presentations of heat to the left and right. We did not analyze responses from animals that were gliding during presentations.

Test of the antennal flagella mediating thermal avoidance

To determine whether the antennal flagella mediated the avoidance manoeuvres, we tested four groups of ten locusts in the manner described above. The control group was flown with no stimulus. Animals in the second group were intact, with the stimulus alternating between left and right. The third group had their antennal flagella removed with scissors one segment above the pedicel. In the last group, the tarsi of the prothoracic legs were severed at the end of the tibia as a control for the surgical manipulation of the previous group. Steering was measured using abdominal transducer signals and is reported as the horizontal displacement (in mm) of the abdomen rather than mean response scores as described above.

Electromyographic recordings from forewing muscles during thermal avoidance

We removed all legs at the coxo-trochanter joint and deafened each animal by destroying both tympanic membranes with an insect pin. Electromyography (EMG) electrodes, which consisted of fine copper wire (0.1 mm diameter) insulated except at the tip, were inserted just beneath the cuticle of the sternum into muscles M97 (forewing first basalars; depressors) and secured with a drop of wax. Activity from the left and right muscles was referenced to a ground electrode, inserted behind the head beneath the pronotum, and was amplified using Grass Instruments (model P15) differential a.c. preamplifiers.

Trials consisted of left and right alternating presentations of heat, with the side of the first presentation chosen randomly. We allowed sufficient time (approximately 30 s) during and between each presentation to allow the temperature to stabilize. The temperature increase for all animals tested (measurements from all presentations, means ± standard deviation) except one was 5.3±0.9 °C (initially 22.7±0.2 °C increasing to 27.9±0.7 °C, N=6). For one animal (three presentations), the temperature increased by 7.5±0.8 °C (initially 26.3±0.1 °C increasing to 33.8±0.7 °C). A photocell placed beneath the suspended animal monitored when the heat lamps were on. Temperature and abdomen position were monitored as described above.

Forewing depressor EMG, abdomen position, temperature and photocell signals were recorded for later analysis onto VCR tape using a Panasonic VCR (model PV-4770-K) and Neuro-corder (model DR-886). We recorded the times of left and right forewing depressor muscle spikes (the first spike if it occurred as a burst) from digitized recordings. Forewing depressor asymmetry was calculated as the difference between the time of the right and left spikes (left time minus right time). Abdomen position and temperature measurements are 50 ms averages taken once every 256 ms from continuous recordings from experimental trials. Spike times and averages were obtained using software and hardware from Datawave Technologies (Longmont, CO, USA). Of 18 animals tested, six (four males and two females) were analyzed for forewing depressor asymmetry. Results from animals that yielded poor EMG recordings or which stopped flying just before or after the heat lamp was turned on were omitted. Similarly, results from animals that turned in only one direction despite

stimulation from both left and right sides (probably because of the rigid tether) were omitted from the analysis.

Statistical analyses

Data were tested for normality and heteroscedasticity and appropriate parametric or non-parametric tests were applied (Zar, 1984). We performed statistical tests using a SigmaStat software package from Jandel Scientific (Corte Madera, CA, USA). All tests assumed significance when $P<0.05$.

Results

Wing movements during thermal stimulation

Animals began to fly when the wind-tunnel was turned on, or when they were lightly touched or startled with an acoustic stimulus (hissing). Results from animals that flew intermittently were not analyzed. The high-speed films showed that during steady, straight flight the left and right forewings and hindwings beat symmetrically, with the forewing wingbeat lagging slightly behind the hindwing wingbeat. The metathoracic legs were held close to the abdomen, which was held straight behind the animal with either a slight dorsi- or ventroflexion. When the heat lamp was presented to one side of the animal, the animal's posture changed in a stereotyped way: the abdomen and hindlegs ruddered away from the side of the heat lamp and the left and right forewings began to beat asymmetrically during the downstroke (Fig. 2). Segments from the analysis of one animal are presented in Fig. 3. When this animal was stimulated with the lamp on the left, the abdomen ruddered to the right. The angle of the forewings, relative to the tether, was asymmetrical during depression (the right

forewing leading the left) but was symmetrical during elevation. The hindwings remained together during the up- and downstroke of the wingbeat. We observed the opposite when the heat lamp was placed on the right.

In some animals (including the one presented in Fig. 3), forewing asymmetry was not zero when the animal was not stimulated. This 'bias' was probably an artefact due to the rigid tether suspending the animal in the wind-tunnel. Results from animals that flew with gross biases were not analyzed.

Abdominal steering

We used abdomen position as an indicator of steering during our trials since abdomen position is a reliable correlate of
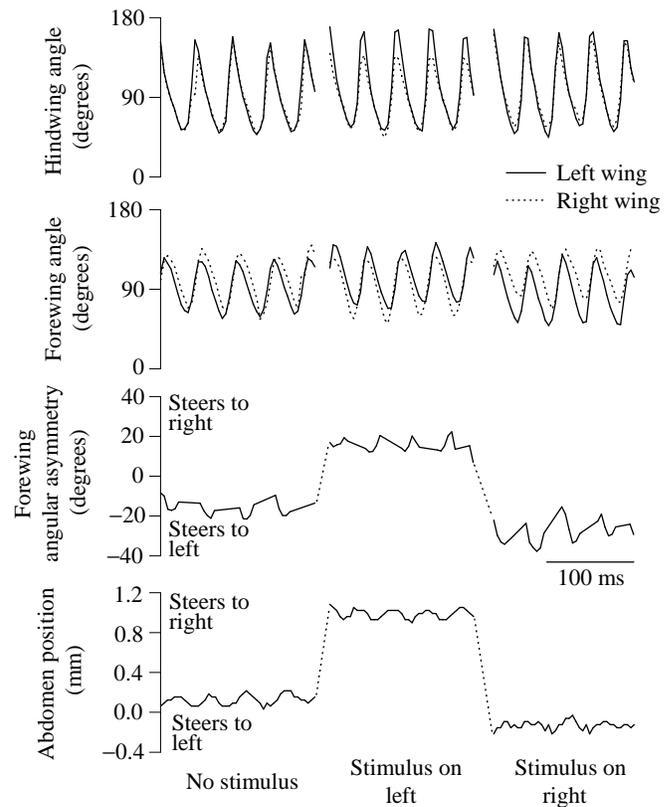
Fig. 3. An analysis of forewing and hindwing kinematics from high-speed motion picture frames taken of a tethered flying locust stimulated with a heat source on the left and right. The abdomen rudders away from the side of the stimulus (lowermost trace) followed by asymmetrical depression of the forewings (middle two traces). With a heat source on the left, the right forewing leads the left during the downstroke, but during the upstroke the left and right forewings are elevated together (second trace from top). The opposite is observed with a heat source presented from the right. Forewing asymmetry is quantified by measuring the difference in wing position during the downstroke (third trace from top). A positive forewing asymmetry indicates a turn to the right, a negative asymmetry indicates a turn to the left. Note that, for this animal, when no stimulus was applied there was a bias in forewing asymmetry to the left which was probably an artefact caused by the tether. Hindwing elevation and depression are usually symmetrical during turning sequences. In the top two traces, 0° is directly beneath the animal and 180° is directly above the animal.
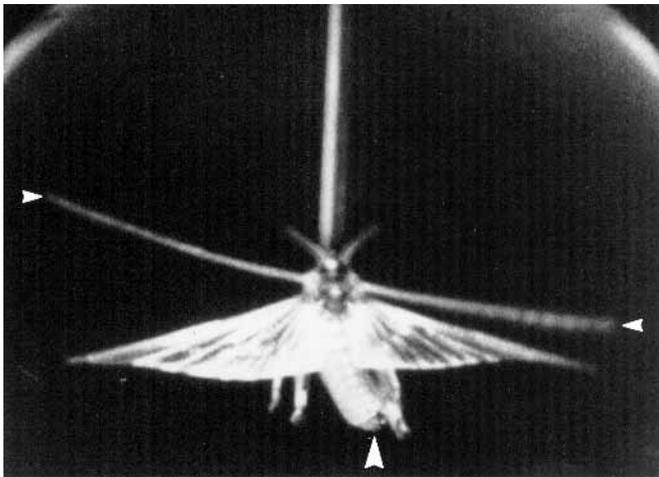

Fig. 2. An image taken from one frame of a trial filmed with the high-speed camera showing the response of a female locust to a thermal stimulus (unfiltered heat lamp) presented on the left. The image, taken during the downstroke, shows greater depression of the right forewing relative to the left, indicating a turn to the right. Note also that the abdomen and hindlegs are ruddered to the right. The circular object in the background is the mouth of the wind-tunnel. Small arrowheads indicate forewing tips; the large arrowhead indicates the tip of the abdomen.
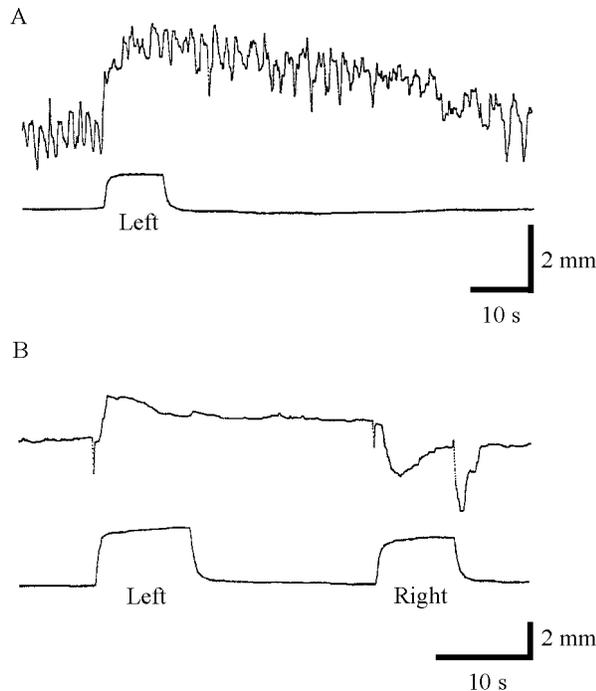
Fig. 4. Recordings from the abdomen position transducer showing abdominal ruddering (steering) away from a heat source. In both A and B, the top trace is the transducer signal and the bottom trace is the stimulus monitor. Abdomen ruddering is always away from the stimulus. The differences in smoothness of the transducer traces in A and B are due to differences in the position of the lateral sensing electrodes, a difference in signal amplification and whether the signal was low-pass filtered. Fluctuations in the transducer signal at stimulus onset in B are electrical artefacts. The change in abdomen position in B when the stimulus on the right was turned off was probably a startle response. Startle responses when the stimulus was turned off were rare.
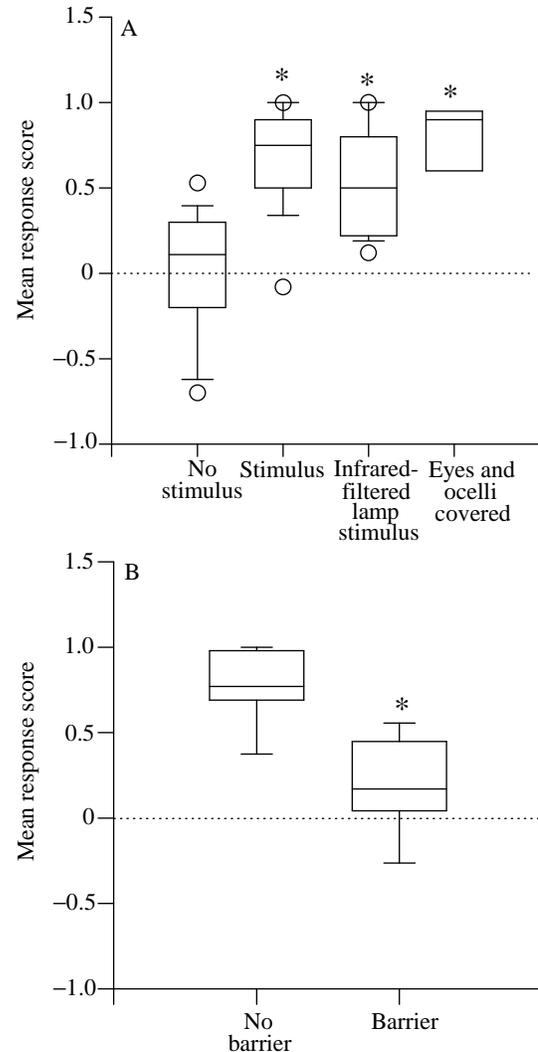

Fig. 5. Box plots of mean response scores (MRSs) for treatments with different test stimuli. Positive MRSs indicate steering away from the stimulus. (A) Responses from animals stimulated with a heat lamp on either the left or right were significantly different (asterisks) from those of animals that were not stimulated; however, MRSs of intact animals, animals exposed only to infrared light (filtered lamp stimulus), and of animals with their eyes and ocelli covered were not significantly different from each other (Kruskal–Wallis one-way ANOVA on ranks, H=22.788, P<0.001, N=17, 14, 14, 4, respectively; Dunn's pairwise multiple comparisons test). (B) MRSs from locusts stimulated with the lamp (emitting visible and infrared light) then with the lamp behind a 12 mm thick acrylic barrier (to attenuate the infrared light) were significantly different (asterisk) (paired t-test, t=3.914, P=0.008, N=7). The line through the centre of the box represents the median; the upper edge of the box represents the third quartile and the lower edge represents the first quartile; the capped bar above the box represents the the ninetieth percentile point and the capped bar below the box represents the tenth percentile point; the circle above the capped bars represents the ninety-fifth percentile and the circle below the capped bars represents the fifth percentile.

steering during flight (Camhi, 1970). Typical traces of abdomen position during thermal presentations are shown in Fig. 4. A stimulus on the left reliably caused flexion of the abdomen to the right; similarly, stimuli on the right caused flexion of the abdomen to the left. The deflection of the tip of the abdomen was typically 10 mm, but deflections up to 20 mm were observed. The heat lamp used in these experiments produces both infrared and visible light. Therefore, to determine which components of the heat lamp were causing the avoidance manoeuvres, we used a combination of infrared transmission filters (that allowed only long-wavelength infrared light to pass) and acrylic barriers (that attenuated the infrared light) (Fig. 5).

When no stimulus was applied, the median mean response score (MRS) was 0.1 (−0.2, 0.3) (first and third quartiles in parentheses respectively). When the heat lamp was turned on, the median MRS was 0.8 (0.5, 0.9), indicating abdominal ruddering away from the lamp. An infrared filter placed in front of the heat lamp still allowed abdominal ruddering away from the lamp with a median MRS of 0.5 (0.2, 0.8). When the eyes and ocelli were covered with opaque nail-polish, to

eliminate visible light as a stimulus to the animal, the median MRS was 0.9 (0.6, 1.0). Locusts flown with the heat lamp stimulus, with the heat lamp filtered or with their eyes and

ocelli covered all showed significantly more abdominal ruddering away from the lamp than locusts without the lamp stimulus [Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, $H=22.788$, $P<0.001$, $N=17, 14, 14, 4$, respectively] (Fig. 5A). Locusts flown with the lamp stimulus then flown again with an acrylic barrier in front of the lamp showed significantly less abdomen ruddering away from the heat lamp in the presence of the barrier (paired t-test, $t=3.914$, $P=0.008$, $N=7$) (Fig. 5B).

Test of the antennal flagella mediating thermal avoidance

In an attempt to determine whether receptors located on the antennal flagella mediated thermal avoidance manoeuvres, we ablated the antennal flagella and tested for directional responses. Animals with and without antennal flagella failed to show directional responses when stimulated with a sham heat source (stimuli from left: t-test, $t=-0.025$, d.f.$=18$, $P=0.98$, $N=10$; stimuli from right: t-test, $t=-0.00166$, d.f.$=18$, $P=0.999$, $N=10$). Abdomen ruddering by intact animals and animals with their antennal flagella removed above the pedicel was not significantly different when the heat lamp was used (stimuli from left: one-way ANOVA, $F=0.587$, $P=0.563$, $N=10$; stimuli from right: one-way ANOVA, $F=1.30$, $P=0.290$,

$N=10$). We are confident that the surgical manipulation did not affect the ability of the animals to respond directionally since the response of animals with amputated prothoracic tarsi was not significantly different from that of those with amputated antennal flagella (Fig. 6).

Electromyographic recordings from forewing muscles during thermal avoidance

The high-speed camera data showed stereotyped wing movements in response to stimulation with a heat source. We recorded electromyographically from the left and right first basalars to determine certain aspects of the motor pattern not evident in the behavioural analyses. Traces from the left and right first basalars during stimulation (Fig. 7) showed (1) a relative increase in burst duration on the contralaterally stimulated side, (2) an overall increase in depressor frequency, and (3) an advancement of contralateral depressor muscle activity relative to that on the stimulated side.
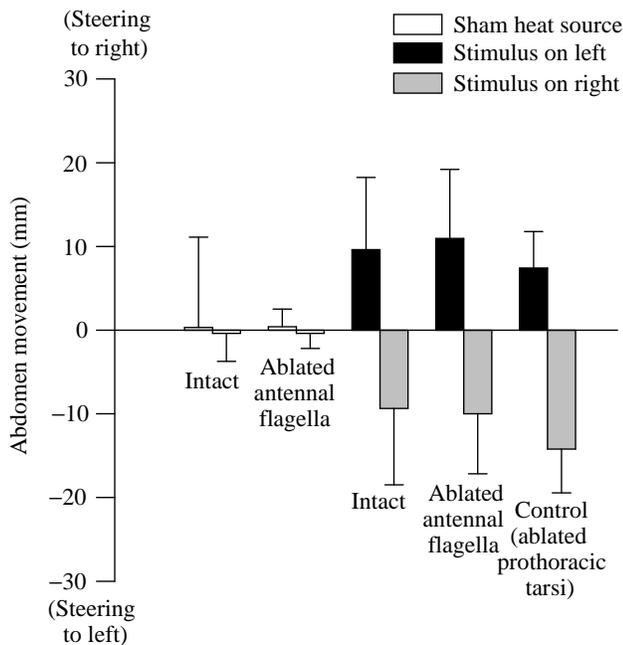

Fig. 6. Locusts with ablated antennal flagella were still able to respond directionally to a heat source with abdominal ruddering (stimuli from left: one-way ANOVA, $F=0.587$, $P=0.563$, $N=10$; stimuli from right: one-way ANOVA, $F=1.30$, $P=0.290$, $N=10$). Animals that were exposed to a sham heat source either when intact or with ablated antennal flagella did not show steering responses (stimuli from left: t-test, $t=-0.025$, d.f.$=18$, $P=0.98$, $N=10$; stimuli from right: t-test, $t=-0.00166$, d.f.$=18$, $P=0.999$, $N=10$). As a control for the ablation procedure, animals with prothoracic tarsi were tested in the same manner as those with antennal flagella ablations, and these animals also responded directionally. Bars and error bars represent means and standard deviations respectively.
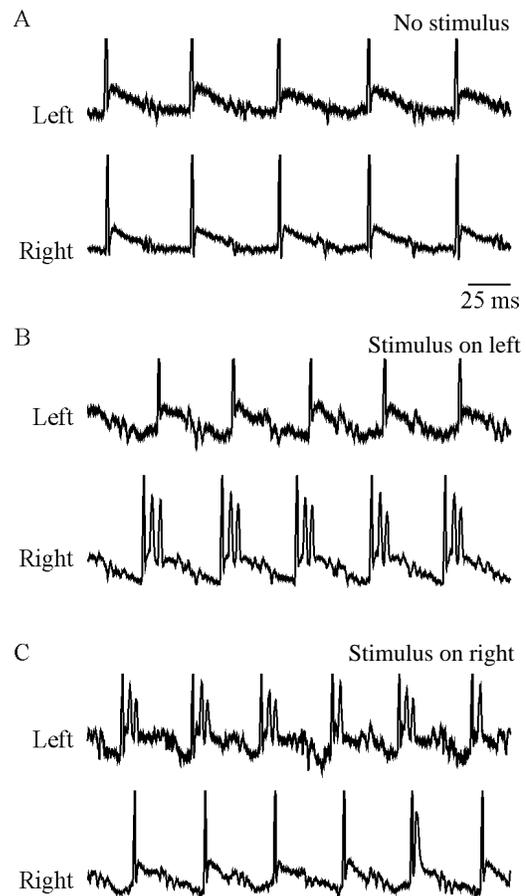

Fig. 7. EMG recordings from the left and right first basalar muscle (M97), a forewing depressor muscle, during tethered flight (A) and during presentation of a heat source to the left (B) or right (C) of an animal. In these traces, taken from the same animal, depressor muscle activity on the inside of the turning path occurred approximately 11 ms before that on the outside of the turning path. Depressor burst duration on the inside of the turning path was greater than that observed on the outside of the turning path. Depressor muscle activation (wingbeat frequency) increased during stimulation.

Burst duration, measured from one trial from each of the six animals tested, on the side opposite the stimulus (the inside of the turn) increased relative to the stimulated side. Recordings of the first basalars from one animal are presented in Fig. 7. When stimulated on the left side (Fig. 7B), the left depressor continued to fire with the same pattern but the right depressor, the muscle activating the wing on the inside of the turn, fired

with a longer burst duration (more spikes per burst). The opposite was observed when the animal was stimulated on the right side (Fig. 7C). Burst durations before stimulation were usually one spike per burst, but in animals with steering biases, two spikes were occasionally observed. Of the six animals included in the EMG analysis, five showed steering biases in which the forewing depressor asymmetry was not zero in the
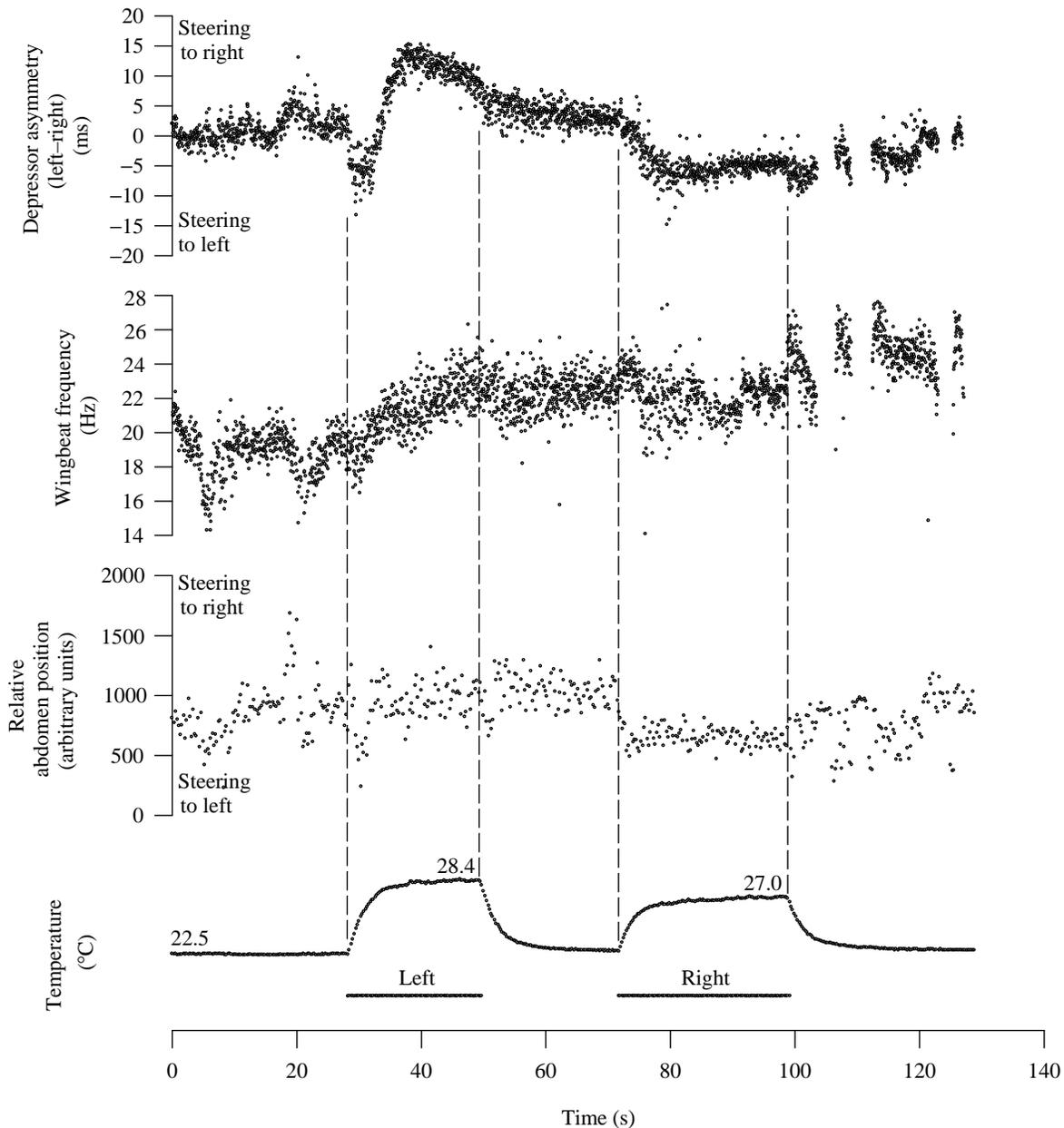

Fig. 8. A time plot of forewing depressor asymmetry, wingbeat frequency and relative abdominal position from one animal. A heat source presented on the left (first stimulus bar) caused a positive forewing depressor asymmetry indicating a turn to the right; the short-latency negative deflection is probably a startle response; a rare occurrence. A heat source presented on the right caused a negative forewing depressor asymmetry indicating a turn to the left. Asymmetries in depressor muscle activation ranged from 6.2 to 19.5 ms with means of 10.3±2.7 ms (stimuli from the left) and 12.0±4.9 ms (stimuli from the right) (mean ± S.D., *N*=6). The lowermost trace indicates the time course of temperature change during the experiment. The stimulus from the right caused abdominal ruddering away from the stimulus, but ruddering in response to the left stimulus is possibly absent because the animal was startled. Depressor muscle activation increased by approximately 3 Hz for all animals tested; however, the presentation from the right is one of the two that showed a marginal decrease in wingbeat frequency; an increase is observed for the left presentation.

absence of a stimulus. The biases were slight, however, ranging from 2 to 5 ms, and we feel that they did not affect the ability of the animals to effect steering manoeuvres.

The frequency of forewing first basalar activation increased during stimulation (an increase in wingbeat frequency) by 2.8±0.8 Hz when stimulated from the left and 3.4±1.2 Hz when stimulated from the right side (values are means ± 1 S.D., N=6). All animals tested showed an increase in depressor frequency; however, during two presentations within separate trials, depressor frequency marginally decreased.

From the behavioural observations, forewing asymmetries during stimulation were the most striking observations. These asymmetries are reflected in the pattern of forewing depressor activation. We refer to asymmetries measured from EMG recordings of the first basalars as forewing depressor asymmetries rather than forewing asymmetries to avoid confusion with those measured from forewing positions. Forewing depressor asymmetries ranged from 10.3±2.7 ms for animals stimulated on the left to 12.0±4.9 ms for animals stimulated on the right (values are means ± 1 S.D., N=6). Depressor asymmetries from one trial (Fig. 7) show that, when the animal was stimulated on the left, the animal's right wing depressor spike preceded that on the left. The change in forewing depressor asymmetry, wingbeat frequency and relative abdomen position recorded for one animal are presented in Fig. 8. When this animal was stimulated on the right, forewing depressor asymmetry indicated that the left first basalar was activated before the right first basalar by approximately 10 ms.

Discussion

A locust that is attempting to change course during flight characteristically shows a skewed posture with the abdomen and hindlegs extended into the turn (e.g. Camhi, 1970; Arbas, 1986; Baader, 1990). In addition, on the inside of the turn there is increased and earlier pronation of the fore- and hindwings (e.g. Dugard, 1967; Baker, 1979; Zarnack, 1988), earlier stroke reversals resulting in asymmetrical phasing of the wingbeat (e.g. Thüring, 1986; Schwenne and Zarnack, 1987; Waldmann and Zarnack, 1988) and a substantially reduced angle between the fore- and hindwing during the downstroke (e.g. Schmidt and Zarnack, 1987; Schwenne and Zarnack, 1987). During simulated collision avoidance, the forewings become strikingly asymmetrical during the downstroke (Robertson and Reye, 1992) coincident with the production of yaw torque towards the side of the lower forewing (Robertson and Johnson, 1993a). All of the above kinematic changes are considered to have the aerodynamic consequence of generating asymmetrical lift and thrust to alter the direction of the flight path.

In the present investigation, we found that shining a heat lamp on one side of a tethered flying locust initiated postural and wingbeat alterations that have most of the above characteristics and that can thus best be interpreted as an attempt by the locust to turn away from the heat source – a thermal avoidance manoeuvre. We did not monitor the timing

or extent of wing pronation during the downstroke. Nevertheless, the lamp-induced generation of asymmetries in the positions of the abdomen and hindleg and in the relative angle of the forewings during the downstroke is consistent with the above interpretation. Indeed, the attempted manoeuvre described here is similar to a collision avoidance manoeuvre known to generate steering torques (Robertson and Johnson, 1993a) and to the manoeuvre associated with negative phonotaxis (R. M. Robertson, D. Robert, K. Dawson-Scully and J. W. Dawson, in preparation). It could be argued that what we observed was the result of non-specific temperature effects on nerve and muscle function on one side of the body, rather than being a thermally stimulated flight manoeuvre. This interpretation is highly unlikely given the nature of the reaction to the heat lamp as a consistent, coherent and coordinated response resembling steering movements initiated by other means. Two other observations lend support to this argument. First, the reactions of the hindwings and forewings were different, with the hindwings showing minimal, if any, asymmetries during the downstroke even though the hindwing mechanisms were equally subject to the direct effects of heat. Second, the major effects on the motor pattern (increase in burst length, advance in timing, see Fig. 7 and below) were on the inside of the turn, i.e. on the *unheated* side of the locust.

The manoeuvre we describe here is associated with relative asymmetries in the timing and strength of the forewing first basalars (earlier and stronger on the inside of the turn) and a moderate increase in wingbeat frequency. It is likely that at least part, and perhaps all, of the increase in wingbeat frequency was an effect of increased thoracic temperature due to direct heating of the animal (e.g. Foster and Robertson, 1992). However, for the reasons outlined above, the asymmetries in the motor pattern would not have been caused by direct effects of increased thoracic temperature. The description of the motor pattern changes is somewhat preliminary, being restricted to a consideration of the activity of only the first basalar muscles of the forewing. Subsequent experiments will investigate the changes occurring in the strength and phasing of the activity of other wing muscles, including those of the hindwing. However, one of the most consistent features of the motor patterning associated with other steering manoeuvres is a shift in the relative timing of the forewing first basalars (Zarnack and Möhl, 1977; Baker, 1979; Thüring, 1986; Schmidt and Zarnack, 1987; Zarnack, 1988; Waldmann and Zarnack, 1988; Hensler and Rowell, 1990). Whereas most of these previous studies describe shifts in the relative timing of first basalar activity of around 5 ms (from as low as 0.1 ms in Thüring, 1986, to around 6 ms in Zarnack and Möhl, 1977), we describe here timing asymmetries during the manoeuvre that average 11 ms, with some single trials giving values of as much as 19 ms. The exception to this is the study of flight steering in a radial wind-tunnel by Baker (1979) in which he described phase asymmetries of up to 13 ms. It is worth underlining that the common feature of the study of Baker and the present study is that, in both, the steering was to effect a change in course

direction rather than to maintain course in the face of external perturbation (a common feature of the other studies). Another similarity between this study and that of Baker is the observation of burst duration increases on the inside of the turn. Course correction manoeuvres generally show little or no correlation with burst duration in the first basalars (Thüring, 1986; Zarnack, 1988; Waldmann and Zarnack, 1988). We believe that the reason for these similarities and differences lies in the fact that the changes in wing kinematics and the underlying motor pattern changes are larger and more obvious when the locust effects a change in course rather than maintains a course.

It is an unfortunate and unavoidable consequence of the experimental constraints that the locust was waxed to a rigid tether. Locusts tend not to generate 100 % lift during tethered flight (Krüppel and Gewecke, 1985), and steering biases in the kinematics and motor patterns are often evident (Möhl, 1985, 1988; Robertson and Reye, 1992; Robertson and Johnson, 1993a). Without exteroceptive feedback to monitor the locomotor consequences of a motor pattern, coordination of the motor pattern and thus the wing movements becomes imprecise. Artificially restoring the feedback removes these biases (Möhl, 1988; Robert, 1988). Examples of this imprecision can be seen in Figs 3 and 8, in which it is evident that forewing angular asymmetry and the asymmetry between the timing of the right and left first basalar EMGs were not always zero in the absence of a heat stimulus. We believe that this tendency to fly with steering biases in the absence of stimuli does not negate our conclusions, although it may have had an effect on the magnitude of the symmetry changes we recorded in response to stimuli.

We observed these steering reactions in response to illumination by a laterally positioned heat lamp. Neither filtering out the visible light with an infrared filter nor covering the eyes and ocelli with opaque paint significantly affected the tendency of a locust to attempt a turn away from the lamp. However, attenuating the infrared component of the radiation with a transparent acrylic barrier significantly reduced the turning tendency, almost to zero. Moreover, the abdominal and motor pattern asymmetries were not immediate, but developed to a maximum over several seconds after the lamp had been turned on, most often without any indication of a startle reaction (upward abdominal flick) associated with the abrupt onset of illumination. When rapid-onset startle components were observed, there was no consistent relationship between the side of illumination and the direction of movement of the abdomen. Finally, it is unlikely that convective thermal gradients, capable of being detected across the locust, would survive in the $3\,\mathrm{m\,s^{-1}}$ air flow from the mouth of the wind-tunnel. For these reasons, we conclude that the locusts were reacting to the infrared component of the stimulus and that the slow onset of the reaction was due to the time inherent in raising the temperature of the cuticular components of the thermosensors.

Although it is clear that insects will orient to thermal stimuli for different reasons (thermoregulation, orientation to prey),

clear experimental demonstrations of thermotaxis have been described rarely, if at all, in insects. The attraction of *Melanophila acuminata* to forest fires *via* infrared detectors (Evans, 1964) implies that they engage in thermotaxis. It was not possible to confirm directly that the heat stimulus was invoking thermotaxis (i.e. locomotion initiated by the stimulus) because of the rigid tether used in our experiments. However, we are confident that the reactions we observed would have resulted in a course change away from the heat stimulus during free flight, and we therefore conclude that the locusts were attempting a thermal avoidance manoeuvre that would result in negative thermotaxis.

Thermotaxis in response to infrared radiation implies an ability by the locust to detect the location of the infrared source *via* bilaterally located sensors with directional sensitivity. An intriguing question raised by this study is that of the nature of the infrared sensors. We anticipated that the thermoreceptors located on the antennal flagella (Waldow, 1970; Altner *et al.* 1981; Ameismeier and Loftus, 1988) would mediate thermotaxis, but removal of the antennal flagella had no significant effect on the thermotactic reactions. Interestingly, Slifer (1951) proposed that crescents of cuticle lacking a cement layer and located tucked behind the frons at the base of each antenna might mediate a directional infrared sensitivity similar to that of crotalid pit vipers. This idea was subsequently refuted by experiments on stationary locusts (Makings, 1964). However, the considerably different physical conditions during flight might warrant a further attempt to assess the role of Slifer's crescents as infrared detectors. Alternatively, the antennal bases, which were not removed in our experiments and which would be appropriately shaded from illumination from the opposite side, may contain the thermosensors. Assuming that the sensors would be best located on the head, a final possibility is that asymmetrical heating of the wind-sensitive head hairs on either side of the head could generate the thermotactic reactions. Miles (1985) has shown that the wind-sensitive head hairs are more sensitive to temperature changes than the mechanoreceptive hairs on the legs and thorax. Furthermore, the temperature sensitivity is transferred to a wind-sensitive interneurone (Miles, 1992). The tritocerebral commissure giant (TCG, Bacon and Tyrer, 1978) is active during flight and is known to mediate compensatory yaw steering (Möhl and Bacon, 1983) in response to the detection of an asymmetrical wind direction. Although temperature effects on wind sensory transmission to the TCG are compensated and the temperature sensitivity of a TCG is minimal when only the surface of the head is heated (Miles, 1992), the idea that asymmetrical heating of the head hairs mediates the thermotaxis we observed should be tested.

It is important to consider what might be the behavioural relevance of this negative thermotaxis. Conceivably, following from the above argument, we have described here a behavioural artefact, i.e. artificial, and asymmetrical, thermal stimulation of the wind-sensitive head hairs may have released a corrective steering reaction which would naturally be released by asymmetrical wind stimulation. In spite of temperature

compensation in the wind sensory pathway, this remains a possibility. Another possibility is that the reaction would prevent a locust from landing on dangerously hot substrata. However, given the relatively long latency for the reaction to develop, it is unlikely that the avoidance manoeuvre would be particularly useful over short distances. A locust would have flown 6–9 m before the reaction had fully developed. A final possibility, and one that we consider has some merit, concerns terrain monitoring from an altitude during longer-term migratory flight. A glance at satellite images of the world from space (Ramanathan *et al.* 1989), or images of more restricted landscapes provided by the thermal infra-red multispectral scanner mounted on an aircraft, readily confirms that large areas of substratum devoid of vegetation are well-defined sources of infrared radiation (O'Neill, 1993). We believe that the negative thermotaxis described here may well provide locusts with an ability to detect, and avoid flying over, the borders of extensive areas of terrain that are dangerously hot and/or barren and thus that have limited food availability. This ability could have considerable survival value for the individuals in a large swarm of locusts migrating long distances.

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