Evidence for idiothetic and allothetic control of thermo-orientation in feather-feeding lice

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A B S T R A C T

Thermal cues are widely used by ectoparasites to find and exploit hosts. Recently, the wing louse Columbicola columbae (Phthiraptera: Ischnocera) was shown to use thermo-orientation when migrating between host microhabitats. Here, we study the control systems governing thermo-orientation by motion tracking louse velocity on spatial and temporal heat gradients. As previously demonstrated, lice placed on spatial heat gradients successfully located nearby heat targets. Unilateral antennectomies were then used to remove spatial aspects of the thermal cue. These lice were still capable of locating heat targets, suggesting their response relied on tracking changes in the cue over time (idiothetic control). Course control was accomplished via angular corrections after louse body-angles deviated from the heat target. Louse behavior on temporal heat gradients provided additional evidence for idiothetic control—lice altered turn size and velocity after temperatures uniformly shifted without any spatial reference. We also show that lice are likely capable of responding to spatial aspects of the cue, consistent with allothetic control. On the spatial heat gradient, lice with two antennae were more efficient at locating heat targets as compared to those with unilateral antennectomies. Additionally, when traveling along temperature isoclines (where lice can detect spatial but not temporal aspects of the cue), lice with two antennae consistently turned towards the heat target, while those with unilateral antennectomies showed no preference. In all, we find evidence that lice can use both idiothetic and allothetic control during thermo-orientation, and likely integrate information from these two systems to guide movements on hosts.

1. Introduction

Host-generated thermal cues are exploited by a wide array of ectoparasites and underlie behaviors central to their survival. For most ectoparasites, such as kissing bugs, bed bugs, mites, ticks and mosquitoes (among others), thermal cues play a key role in locating and initiating contact with hosts (Bowen 1991; Owen & Mullens, 2004; Lehane, 2005, Lazzari, 2009; Boxler et al., 2016). Once hosts are acquired, detecting and responding to thermal cues is central to their ability to exploit hosts. For example, tracking thermal gradients on the host can facilitate navigation between preferred host regions (Halbritter & Mullens, 2011; Harbison & Boughton, 2014). Additionally, thermal cues may help parasites identify key resources, such as the presence of a nearby blood vessel (Ferreira et al., 2007) or the location of host regions with optimal temperatures for the development of eggs or larval stages (Murray, 1956a,b).

Thermo-orientation underlies many important parasite behaviors, it is of interest to better understand how they sense thermal cues, store and recall this information and ultimately generate behavioral responses. Much progress has been made in this regard. The location and prevalence of thermosensilla in antennae and the associated neural anatomy have been reported for many species (Wigglesworth, 1941; Klompen & Oliver, 1993; Gingl et al., 2005; Crespo & Vickers, 2012). Additionally, behavioral responses to thermal cues (e.g. changes in velocity, turning behavior) have been documented in a number of parasites (Wigglesworth & Gillet, 1934; Wigglesworth, 1941; Marshall, 1981; Lehane, 2005; Kilpinen & Mullens, 2004; Owen et al., 2005; Ferreira et al., 2007; Lazzari, 2009).

Fewer studies have focused on the control systems governing parasite thermo-orientation. After thermosensilla receive the signal, parasites must perceive the heat cue by filtering and integrating this information in their central nervous systems prior to generating behavioral responses. Perception of the cue can be accomplished through either allothetic control of orientation using external information, or idiothetic control using internally stored information (Mittelstaedt et al., 1979; Visser, 1988). In allothetic control, paired receptors (e.g. antennae) are used to detect spatial asymmetries in the stimulus intensity. A desired course would then be maintained by turning towards
or away from the more stimulated side.

Alternatively, orientation using idiothetic control requires that parasites use internally stored information to guide movements (e.g. mechanosensory information and/or proprioceptive feedback). For example, a parasite could track changes in temperature over time as well as information on their body orientation or recent turns. Integrating this information would enable the parasite to make corrective movements that maintained a desired direction (e.g. altering velocity or changing turning behavior) or even enable a return to its starting point (Visser, 1988; Mittelstaedt, 2000). Orientation using idiothetic control thus enables directed movement without using spatial aspects of the cue and can be accomplished even using a single temperature receptor.

However, it is difficult to experimentally distinguish which control system guides orientation, as the path traveled using either allothetic or idiothetic control might appear similar. Further complicating matters, parasites may integrate information from both allothetic and idiothetic control systems to guide movement, or switch between systems as environmental conditions or internal states change (Visser, 1988; Lazzari, 2009).

In this study, we explore the control systems governing thermo-orientation in the wing louse, *Columbola columbia* (Phthiraptera: Ischnocera), a small insect (~2 mm long) that infests Rock Doves (*Columbia livia*). Wing lice are permanent parasites that cement their eggs to, and spend their entire lives on the host’s feathers. Wing lice show strong microhabitat specificity—up to 90% of individuals are found on bird flight feathers (wings and tail) where they insert their elongated bodies between coarse feather bars to escape host preening (Bush & Maleinke, 2008). However, wing lice must repeatedly migrate from the flight feathers to bird body regions to feed on the insulating barbs of downy feathers.

Temperature has been shown to strongly influence many life-history traits in other louse species, such as their distribution on hosts (Brown, 1970; Halbritter & Mullenks, 2011), placement of eggs (Murray, 1956a, b), and seasonal movements on hosts (Murray, 1990). Detecting temperature variations on the host is similarly important in wing lice. Early work with *C. columbiae* demonstrated that they respond to thermal cues and prefer temperatures ranging from 32 to 38 °C (Conci, 1952; Rakshpal, 1959), similar to those found on the host (Harbison & Boughton, 2014). More recent work has shown that wing lice are adept at thermo-orientation on spatial heat gradients, and can successfully locate nearby heat targets that approximate host temperatures. Additional heat choice assays showed that wing lice can use host-generated thermal cues to guide their repeated migrations between the cooler flight feathers where they hide from preening and the warmer body regions where they feed (Harbison & Boughton, 2014). Thermal cues may also be important in louse microhabitat selection, as different louse life stages (eggs, nymphs and adults) tend to reside in bird regions that provide optimal temperatures for their growth (Harbison & Boughton, 2014).

Here, we study the control systems guiding thermo-orientation using motion tracking to analyze the movements of wing lice on heat gradients. To determine whether they are capable of idiothetic or allothetic control, we focused on situations where lice were only capable of using a single control system. This was accomplished using unilateral antennectomies to prevent lice from detecting spatial aspects of the cue, and by placing lice in temporal heat gradients where they experienced uniform temperature shifts over time without any spatial reference.

2. Materials and methods

2.1. Spatial heat gradient

Previous research demonstrated that lice placed on arenas with spatial heat gradients would exit the arenas near the heat targets (Harbison & Boughton, 2014). Here, we used the same apparatus to track the movements of lice throughout trials in order to examine the control mechanisms governing thermo-orientation. The gradient was created on a 3 cm diameter circular filter paper arena affixed to a glass Petri dish with double-sided tape (see Harbison & Boughton, 2014). This apparatus was elevated with 2 metal blocks—the first stabilized the Petri dish, and the edge of the second was slid 2-3 mm under the Petri dish directly beneath the “target” area of the arena (denoted 0’). This second block was heated using a Kapton® heating mat connected to a Digisense® temperature controller. A thermocouple probe was secured at the 0° mark and connected to the controller to precisely maintain the target temperature. This generated a radial heat gradient that ranged from 37 °C at the heat target (approximating host temperature) to 24 °C at the opposite end of the arena.

For each trial, a single adult louse was released in the center of the arena and the trial continued until the louse reached the edge of the arena. The initial orientation of the louse relative to the heat target was alternated 90° in each successive trial. Louse movements were filmed using a Proscope HR (Bodelin Technologies) mounted 10 cm above the arena. To enable motion tracking, a pin was used to mark each louse with two white fabric paint dots (Scrubbies®) on the anterior and posterior regions of their abdomen prior to the trial.

Lice placed on the spatial heat gradient were subject to one of three treatments (n = 40 lice per treatment): partial unilateral antennectomy, partial bilateral antennectomy, or a small surgical ablation to the cuticle near the posterior end of the abdomen (sham control). Each antennal ablation removed the 2 distal segments (flagellomeres) that house tactile sensilla and ‘tuf’ organs that are thought to function as thermo-hygrosensilla (Altner et al., 1983; Clarke, 1990; Steinbrecht, 1994; Smith, 2001; Crespo & Vickers, 2012). Tuft organs have been reported on the distal flagellomeres of various louse species (reviewed in Smith, 2001), however their role as thermo-hygrosensilla has not yet been experimentally tested. We are not aware of reports of thermo-hygrosensilla on other louse body regions. Ablations were performed under a dissecting scope using Vannas spring scissors (2.5 mm cutting edge; FST®). To reduce movement while applying paint markers and removing antennal segments, lice were temporarily placed on filter paper sitting atop an ice-cooled metal plate, and then returned to recovery arenas prior to trials.

As additional controls, separate lice were presented with arenas of uniform temperature; either room temperature (21 °C) or host temperature (37 °C). The latter temperature was achieved by placing the apparatus on a glass tile sitting atop a large heating block (see Harbison & Boughton, 2014). A thermocouple probe was affixed to the arena and connected to the temperature controller to maintain constant temperature.

2.2. Temporal heat gradient

We designed an assay to determine whether lice can use internally stored information (idiothetic control) to respond to temperature shifts over time without spatial reference to the heat source (e.g. Owen et al., 2005). To accomplish this, the bottom of a Styrofoam cylinder was replaced with tinfoil, and a 5 cm diameter circular filter paper arena was affixed to the tinfoil base. The entire apparatus was suspended in a beaker so it was partially submerged in pre-heated water, which kept the arena at 32 °C (corresponding to temperatures found on flight feathers; Harbison & Boughton, 2014). For each trial, a louse marked with two fabric paint dots (see Section 2.1) was placed in the center of the arena and allowed to acclimatize for 30 s. Louse movement was then filmed using a Proscope HR (Bodelin Technologies) affixed above the apparatus. Precisely 8 s after filming began, 200 mL of heated water was gently added to the bath, which uniformly changed the arena temperature to 36 °C but did not create a spatial heat gradient along the horizontal plane of the arena. This treatment approximated a shift from temperatures associated with the bird flight feathers (their preferred regions), to those found on body regions (Harbison & Boughton, 2014). A second treatment (36 °C → 32 °C) simulated the opposite temperature
shift, and a third treatment (32 → 32 °C) served as a control to ensure that the addition of water did not impact louse behavior. These treatments allowed us to observe louse behaviors without deviating from temperatures they would normally experience on the host.

Lice for the spatial and temporal heat gradients were collected from a culture stock maintained on wild-caught Rock Doves kept on a 12:12 L:D photoperiod. Lice were placed on a feather in a holding container for 1–2 h prior to trials. Each experimental apparatus was placed on a 2.5-cm-thick honeycomb optical breadboard (Newport) to minimize vibration, and trials were carried out at the same time of day in a quiet room with dim uniform lighting (8–10 lx), an ambient temperature of 21 °C, and a relative humidity between 30 and 40%. All experiments used adult male and female lice. A new Petri dish, filter paper arena and louse were used for each trial.

2.3. Data analysis

Digitizing louse paths. The program MaxTRAQ (Innovision Systems, Inc.) was used to digitize the positions of the two dots painted on the louse’s abdomen, which was then converted to Cartesian coordinates. The distance from the louse to the edge of the arena (at the 0° mark) was also calculated throughout trials as well as the body-angle of the louse relative to horizontal and relative to the heat target (when present).

Prior to analysis, the path of each louse was broken into “steps” that correspond to the amount of time it takes lice to move one body length (2 s or every 8th frame), as recommended by Tourtellot et al. (1991). This method minimizes noise generated from aspects of the animal’s movements not related to thermo-orientation (i.e. body “wobble”) and generates more meaningful measures of angular displacement as compared to higher resolution sampling.

Spatial heat gradient. Body-angle relative to the 0° mark at every step (each 2 s) was used to calculate the average louse bearing (ALB) for the entire trial. Additionally, when each louse reached the edge of the arena, the angle of the endpoint relative to the 0° mark was recorded as per Harbison & Boughton (2014). The ALBs and endpoint angles were pooled for each treatment and an r-value was calculated for each measure, which indicates how tightly points clustered around the average angle (Batschelet, 1981; 0 ≤ r ≤ 1, with values approaching one indicating tighter clustering around the average angle). In heat gradient treatments, V-tests were used to determine whether louse ALBs and endpoint angles were associated with the heat target at 0° (Batschelet, 1981). For controls on arenas of uniform temperature, Rayleigh tests were used to determine whether ALBs and endpoint angles differed from a random distribution (the V-test could not be used because there was no predicted direction of movement).

Linear velocity was calculated between sequential steps throughout trials using trigonometry to determine the change in position over time of the anterior dot on the louse’s abdomen. Mean velocity was compared between all treatment groups using ANOVA and post-hoc Tukey-Kramer tests. Additionally, for heat gradient treatments, Kendall rank correlations were used to test for correlations between velocity and distance from the heat target.

Turns were defined as angular displacements of ≥30° between steps. Turn frequency was calculated by dividing the number of turns taken by the length of the trial. Mean turn size and turn frequency were compared between treatment groups using ANOVA and post-hoc Tukey-Kramer tests. To determine whether turn frequency changed throughout trials, we split the first 20 s of each trial into 5 s blocks and recorded the percentage of turns taken in these four time-blocks. The values for each block were averaged within treatment groups then compared to a 1:1:1:1 ratio using a χ² test to determine if lice turned more often than expected during any given time-block. The 20 s window was chosen to maximize sample size, as lice began to reach the arena edge after this timeframe. Additionally, for heat gradient treatments Kendall rank correlations were used to test for correlations between turn size and distance from the heat target.

Two measures were calculated to estimate whether lice executed directed or tortuous paths. We first calculated the angular deviation from the ALB at each step (the difference between their body-angle and their ALB), with smaller mean deviations from the ALB throughout trials indicating a more directed path. Next, for each louse, we calculated an r-value indicating how tightly their body-angles at each step clustered around the ALB, with higher r-values indicating a more directed path. Mean deviations from the ALB and r-values were pooled for each treatment group and compared using ANOVA and post-hoc Tukey-Kramer tests.

To determine whether lice were capable of correcting their heading relative to the movement of their body, we tested for correlations between the size of a louse’s angular deviation from its ALB and the size of their subsequent angular movement after 2 steps (e.g. Owen et al., 2005). This timeframe was chosen as louse angular movements did not occur until 3–4 s after temperatures shifted in the temporal heat gradient (see Section 3.2). Movements back towards the ALB were categorized as “returns” while movements away from the ALB were “departures.” We restricted our analysis to trials where r-values for body-angles relative to the ALB were ≥50 to include only lice engaging in directed movements, and to steps for which the louse deviated from the ALB by ≥30° to maximize the chance of observing lice engaging in corrective turning (small deviations from the ALB would presumably not require any corrections in their bearing). The ALB was used as a proxy for the heat target to enable comparisons between lice in the heat gradient treatments and those placed on control arenas of uniform temperature. ALBs tightly clustered around the heat target for lice with two antennae (see Section 3.1), indicating the ALB was a good proxy for orientation towards the heat target.

Finally, to determine whether lice can detect spatial asymmetries in the heat stimulus using paired antennae, we focused on instances where louse body-angles were within 15° of perpendicular relative to the heat target (75–105 and 255–270°), then determined the size and number of subsequent angular movements after 2 steps. We categorized these movements as returns towards the heat target or departures, and compared the size of returns/departures with t-tests and the number of returns/departures using χ² tests (this analysis was not done for lice in 37 and 21 °C control groups as there was no heat target present).

Temporal Heat Gradient. To determine the response of lice to temporal shifts in temperature, their velocity and angular deviation was tracked from 8 s prior to the temperature shift to 10 s post-shift. Repeated-measures ANOVAs were used to compare mean velocity and angular displacement among treatment groups. Post-hoc repeated-measures ANOVAs were additionally used for pairwise comparisons between treatment groups.

3. Results

3.1. Spatial heat gradient

Orientation on the heat gradient. Previous work demonstrated that lice placed on spatial heat gradients orient to and accurately locate nearby heat targets (Harbison & Boughton, 2014). Lice in the current experiment (with two intact antennae) were similarly adept at thermo-orientation on identical heat gradients (Figs. 1A & 2A). Similar to the previous study, louse endpoint angles upon exiting arenas showed significant clustering around the heat target (Fig. 2A, V-test, r = 0.69, p < 0.0001). Lice were additionally motion tracked in the current study enabling a more detailed examination of behavior throughout trials. The average louse bearings (ALB) of lice with two antennae throughout trials showed significant clustering around the heat target (Fig. 2A, V-test, r = 0.62, p < 0.0001). Lice with unilateral antennectomies also showed significant clustering of ALBs (Figs. 1B & 2B, V-test, r = 0.26, p < 0.05) and endpoint angles (V-test, r = 0.27, p < 0.05) around the heat target, however their accuracy was reduced.
**Velocity.** Lice typically walked continuously throughout each trial and rarely stopped or displayed rapid changes in velocity. However, mean velocity (±SE) differed significantly between treatment groups (Fig. 3A; ANOVA, F = 29.8, df = 4, p < 0.0001), with lice in the 37 °C control traveling significantly faster on average (0.75 ± 0.05 mm/s) than any other treatment group (Tukey-Kramer, p < 0.05), and over twice as fast as lice in the slowest treatment group (21 °C control; 0.25 ± 0.02 mm/s). For lice placed on heat gradients, those with two antennae traveled at a similar speed to those with bilateral antennectomies (Fig. 3A; Tukey-Kramer, p > 0.05), and significantly faster than those with unilateral antennectomies (Tukey-Kramer, p < 0.05).

The amount of time it took lice to reach the edge of the arena also differed among treatment groups (Fig. 3B; ANOVA, F = 14.1, df = 4, p < 0.0001). Not surprisingly, lice that traveled at a greater average velocity also tended to reach the arena edge sooner than slower lice. For example, lice with unilateral antennectomies and those in the 21 °C control traveled at a lower velocity than other groups and took 2–3 times longer to reach the arena edge as compared to lice in the heat gradient treatment with two antennae and those in the 37 °C control (Fig. 3B; Tukey-Kramer, p < 0.05). However, lice in the heat gradient treatment with two antennae reached the edge in a similar time to those in the 37 °C control (Tukey-Kramer, p > 0.50) despite traveling at a significantly slower velocity (Tukey-Kramer, p < 0.05). This likely reflects the straighter paths of lice orienting to heat targets as compared to the more tortuous paths of those in arenas of uniform temperature (see 'path profile' below).

When a heat gradient was present, we also determined whether velocity changed as lice approached the heat target. For lice with unilateral antennectomies, mean velocity (±SE) decreased significantly as the heat target was approached (Kendall Rank Correlation, τ = −0.31, p < 0.0001), from 0.14 ± 0.01 mm/s, on average, when lice were 25–30 mm from the target to 0.33 ± 0.03 when 0–5 mm away. In contrast, there was no correlation between louse velocity and distance from the heat target in treatments where lice had two antennae (Kendall Rank Correlation, τ = −0.02, p = 0.40) or bilateral antennectomies (Kendall Rank Correlation, τ = −0.07, p = 0.17).

**Turning behavior.** Turns were defined as a change in angular position ≥30° between steps to avoid measuring small angular movements not related to turning behavior (see Section 2.1). Overall, there was a significant difference among treatment groups in their turn frequency...
turns throughout trials, and showed no signi-
ificant difference (Tukey-Kramer, p < 0.05).
In contrast, turn frequency did not differ
significantly for lice in the unilateral anten-
nectomy, bilateral anten-
nectomy, and 37 °C control treatments (Tukey-Kramer, p > 0.05).

The average size of turns throughout trials also differed between
treatments (Fig. 4B; ANOVA, F = 6.5, df = 4, p < 0.0001). Again, this
was largely driven by lice with two antenna in the heat gradient
treatment and lice in the 21 °C controls that took significantly smaller
turns, on average, as compared to lice in other treatment groups
(Tukey-Kramer, p < 0.05). For heat gradient treatments, we further
tested for changes in turn size as the heat target was approached. There
was no correlation between turn size and distance to the heat target for
lice with two antennae, unilateral antennectomies, or bilateral anten-
nectomies (Kendall rank correlation, $-0.03 \leq r \leq 0.12$, p > 0.17 for
all comparisons).

Lice with two antennae in the heat gradient treatment differed in
when they turned during trials. Typically, they executed a few large
initial turns, which roughly oriented their bodies towards the heat
target. Then, subsequent turns were less frequent and angular changes
were smaller as they moved towards the heat target. We tracked the
number of turns taken during 5 s increments for the first 20 s of trials.
Lice with two antennae on heat gradients took significantly more turns
than expected in the first 5 s (62% of all their turns) when compared to
an even distribution of turns throughout trials (Fig. 4C; $\chi^2 = 74.8$,
p < 0.0001). Lice in all other treatment groups consistently executed
turns throughout trials, and showed no significant bias in when turns
occurred ($3.4 \leq \chi^2 \leq 10.6$, p > 0.05 for all comparisons). Twenty
seconds was chosen for this comparison as many lice reached the arena
echo soon after this time period.

Path profile. To determine whether lice executed paths that were
directed or more tortuous, we first determined the mean angular
deviation of each louse from its average bearing (ALB) throughout the
trial. After pooling this data for each treatment group, we found a
significant difference in the mean deviation of lice from their ALBs
between all treatment groups (Fig. 5A; ANOVA, F = 28.3, df = 4,
p < 0.0001), which was again driven by the strikingly
larger r-value of lice with two antennae in the heat gradient treatment
as compared to other groups (Tukey-Kramer, p < 0.05). Lice from all other groups showed a similar
degree of deviation from their ALBs throughout trials (Tukey-Kramer,
p > 0.05).

The path profile can also be characterized for each louse by de-
termining whether their body-angles at each step clustered around their
ALB throughout the trial (as measured by the r-value, with larger r-
values indicating a straighter path). There was a significant difference
in the mean r-value between treatment groups (Fig. 5B; ANOVA,
F = 6.5, df = 4, p < 0.0001), which was again driven by the strikingly
larger r-value of lice with two antennae in the heat gradient treatment
as compared to other groups (Tukey-Kramer, p < 0.05). Among the
remaining groups, there was little difference in mean r-values (Tukey-
Kramer, p > 0.05).

Course control. To determine whether lice engage in corrective turns
to maintain a bearing towards the heat target, we tested for correlations
between the angular deviation from the ALB, and the size of subsequent
angular movements either back towards the ALB (returns) or away from
the ALB (departures). Lice on heat gradients with two antennae, uni-
lateral antennectomies, and those in 37 °C controls showed significant
positive correlations between the size of their angular deviation from
the ALB and the size of subsequent returns towards the ALB but not
subsequent departures (Table 1).

In contrast, lice on heat gradients with bilateral antennectomies and
those in 21 °C controls showed significant positive correlations between
their angular deviations from the ALB and subsequent returns and de-
partures.

Finally, to determine whether lice are capable of detecting and

Fig. 3. The mean velocity of lice throughout trials
(A) and the mean time it took to lice to reach the
edge of arenas (B) after they were placed on spatial
heat gradients or control arenas of uniform tem-
perature. Letters indicate which treatment groups
have significantly different means (ANOVA with
post-hoc Tukey-Kramer tests, p < 0.05).
responding to temperature differences between their paired antennae, we focused on instances where louse body-angles were within 15° of perpendicular relative to the heat target (75–105 and 255–295°). At this angle, the heat source was to their side and remained at a constant distance during movement. While walking along these temperature isoclines, their thermal environment would not change as they moved. However, differences in temperature on either side of the louse could still be detected using paired antennae. We compared the number and size of any angular movements of the louse relative to the heat target after their bodies had been perpendicular to the heat target. Lice with two antennae showed a marked preference for engaging in angular returns towards the heat target (65% of subsequent movements) as compared to departures (35%) after body-angles were perpendicular to the target (χ² test, p < 0.0001). Furthermore, the mean size (± SE) of these returns (14.9 ± 1.3°) was significantly greater than those of departures (10.3 ± 1.3°; t-test, t = 5.2, p = 0.0001). In contrast, lice with unilateral or bilateral antennectomies did not differ in the number of returns or departures that were taken after perpendicular to the target (51:49%, χ² = 0.15, p = 0.22 for unilateral antennectomies; 52:48%, χ² = 0.56, p = 0.46 for bilateral antennectomies) and showed no significant difference in the size of their returns and departures (t-test, t = 1.5, p = 0.22 for unilateral antennectomies; t = 1.1, p = 0.30 for bilateral antennectomies).

3.2. Temporal heat gradient

Velocity. There was a significant difference in velocity among treatment groups over the course of the trials (Fig. 6A; Repeated-measures ANOVA, F = 10.48, num df = 9.5, den df = 414, p < 0.0001). Post-hoc pairwise comparisons indicate this difference was driven by strong changes in velocity post-temperature shift in both experimental treatments—a roughly 2-fold decrease in velocity by lice in the 36 → 32 °C treatment, and a more moderate but significant increase in velocity of lice in the 32 → 36 °C treatment as compared to controls (Repeated-measures ANOVA, 36 → 32 vs. 32 → 32 °C, F = 9.1, num df = 4.9, den df = 283, p < 0.0001; 32 → 36 vs. 32 → 32, F = 4.9, num df = 4.4, den df = 254, p = 0.0005). Lice in the 32 → 32 °C control showed little change in velocity throughout the trial, indicating the removal and addition of water to the apparatus had little effect on louse velocity.
Angular displacement. There was a significant difference among treatment groups in their angular displacement over the course of the trials (Fig. 6B; Repeated-measures ANOVA, $F = 3.1$, num df = 13, den df = 578, $p = 0.0002$). Post-hoc pairwise comparisons indicate this difference was entirely driven by a roughly 2-fold increase in the angular displacement of lice in the 32 → 36 °C treatment as compared to controls (Repeated-measures ANOVA, $F = 4.0$, num df = 6.1, den df = 354, $p = 0.0007$). In contrast, angular displacement in the 36 → 32 and 32 → 32 treatments showed little variation throughout trials, and there was no significant difference in displacement between these treatment groups (Repeated-measures ANOVA, $F = 0.5$, num df = 5.9, den df = 340, $p = 0.77$).

4. Discussion

To characterize the control systems governing thermo-orientation, we analyzed the behavior of lice placed on either spatial or temporal heat gradients. Lice unable to detect spatial aspects of the thermal cue were still capable of thermo-orientation (i.e. lice with unilateral antennectomies placed on spatial heat gradients as well as those with two antennae on temporal heat gradients). This shows that thermo-orientation can be accomplished by tracking temporal changes in the cue, consistent with idiothetic control. However, we also found that lice are capable of detecting and responding to spatial differences in temperature between antennae, consistent with allothetic control. Thus, lice appear capable of using both idiothetic and allothetic control to direct their movements on heat gradients. Further work will help clarify the circumstances when each control system is used and how lice integrate information from both control systems during thermo-orientation.

4.1. Spatial heat gradients

Directed movement. Lice with two antennae successfully oriented to nearby heat targets—over 70% of lice reached the arena edge within a ± 60° arc of the heat target. These results were consistent with a previous study using the same apparatus (Harbison & Boughton, 2014). The current study additionally employed motion tracking to enable a more detailed examination of louse behavior throughout trials. While moving in the arena, the body-angles of lice with two antennae were significantly clustered around the heat target, suggesting some form of course control was employed.

Lice with unilateral antennectomies were also capable of directed movement toward heat targets demonstrating thermo-orientation is possible without the use of paired antennae. However, these lice were less adept at accurately locating heat targets as compared to lice with two antennae, indicating the use of paired receptors enables more efficient thermo-orientation (r-values for both louse body-angles throughout trials and endpoint angles when exiting the arena were over two-fold lower for lice with unilateral antennectomies as compared to those with two antennae). In contrast, lice with bilateral antennectomies showed a random dispersion of body-angles throughout trials and endpoint angles indicating antennae are necessary for thermo-orientation. For control lice placed on arenas of uniform temperature, body-angles and endpoint angles were also randomly dispersed around the arena. These data additionally provide experimental support for the proposed location of thermosensilla on the terminal antennal segment of lice (Wigglesworth, 1941; Clarke, 1990; Steinbrecht, 1994; Crespo & Vickers, 2012).

Angular course control. Motion tracking lice throughout trials enabled a more detailed examination of how lice maintain paths directed towards the heat target. One possibility is the use of angular course control, whereby angular deviations from a desired bearing are followed by corrective angular movements. We tested for this possibility by comparing the size of angular deviations from the average louse bearing (ALB) to the size of any subsequent angular returns or departures relative to the ALB. The ALB served as a proxy for the direction

Table 1
Comparing the magnitude lice deviated from their average bearing to the size of their subsequent angular movements towards (returns) or away from (departures) their average bearing. Bold font denotes a significant correlation (Kendall τ).

<table>
<thead>
<tr>
<th></th>
<th>Two antennae</th>
<th>One Antenna</th>
<th>No Antenna</th>
<th>37 °C Control</th>
<th>21 °C Control</th>
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<tbody>
<tr>
<td>Returns</td>
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<tr>
<td>Kendall τ</td>
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<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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<tr>
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<td>$P$</td>
<td>0.85</td>
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<td>0.004</td>
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of the heat target (ALBs of lice with two antennae and unilateral antennectomies showed significant clustering around the heat target), and additionally enabled comparisons between lice on heat gradients to those on control arenas lacking a heat target.

The size of angular deviations from the ALB for lice with two antennae as well as those with unilateral antennectomies were positively correlated to the size of subsequent returns, but not departures. This pattern is consistent with lice engaging in corrective turns after they had deviated from a path directed towards the heat target. This method of course correction has been observed in other systems as well, most notably in parasitic mites that were observed to execute similar angular corrections when placed on spatial heat gradients (Owen et al., 2005).

In contrast, lice with bilateral antennectomies as well as those in 21 °C controls showed significant correlations between their deviation from the ALB and both their returns and departures. When lice were unable to detect a heat gradient (either because of antennectomies or being placed on a room temperature arena) they likely reverted to a simple search behavior and alternated turning from one side to another (e.g. Fig. 1C & E). Their circuitous paths could be explained by an internal asymmetry that introduced a slight bias towards one side. This type of circling in the absence of external stimulation has been observed in numerous systems (Preiss & Kramer, 1986; Wendler & Scharstein, 1986; Visser, 1988).

Unexpectedly, control lice on uniformly heated arenas (37 °C) traveled nearly 3× faster, on average, than control lice on arenas at ambient temperature (21 °C), which likely reflected a switch to more active host seeking behavior on arenas approximating host temperature. The velocity of lice placed on spatial heat gradients fell in between these two control groups and did not differ greatly between treatment groups. Interestingly, lice with unilateral antennectomies showed an increase in velocity as the heat target was approached; average velocity was over 3× greater when lice were within 5 mm of the target as compared to that when they were 25–30 mm away. This suggests that lice with unilateral antennectomies tracked changes in stimulus strength over time, consistent with idiothetic control. In contrast, lice from all other treatment groups showed a more even distribution of turns throughout trials. For example, focusing only on the first 20 s of each trial, 62% of turns occurred in the first 5 s for lice with two antennae, as compared to 31–37% for all other treatment groups.

Velocity. Control lice in uniformly heated arenas (37 °C) traveled nearly 3× faster, on average, than control lice on arenas at ambient temperature (21 °C), which likely reflected a switch to more active host seeking behavior on arenas approximating host temperature. The velocity of lice placed on spatial heat gradients fell in between these two control groups and did not differ greatly between treatment groups.

Turning behavior. While turning behavior was similar in most treatment groups, lice with two antennae on heat gradients turned 2–3× less often throughout trials and their turns were 15–21° smaller, on average, than other groups with the exception of 21 °C controls. As the presence of paired antennae likely resulted in more efficient orientation, fewer and smaller angular corrections were needed. This pattern is also consistent with the differences in turn frequency over time between treatment groups. Lice with two antennae on heat gradients typically initiated a series of larger initial turns immediately after being placed on arenas then reduced turn frequency after acquiring a general heading towards the target. In contrast, lice from all other treatment groups showed a more even distribution of turns throughout trials. For example, focusing only on the first 20 s of each trial, 62% of turns occurred in the first 5 s for lice with two antennae, as compared to 31–37% for all other treatment groups.

4.2. Temporal heat gradients

When placed on spatial heat gradients, lice with unilateral antennectomies were still capable of locating the heat target suggesting temporal, rather than spatial properties of the stimulus were used in orientation. To further test this possibility, we tracked the movement of
lice with two antennae on arenas while temperatures shifted uniformly in the plane of louse movement. Lice were subject to temperature shifts that either mimicked movement towards (32 → 32 °C) or away from flight feathers (32 → 36 °C), the region where lice spend the large majority of their time and seek shelter from bird preening.

As the temperature shift mimicked movement away from flight feathers (32 → 36 °C), lice responded by nearly doubling their mean (± SE) angular displacement (from 33.1 ± 6.2° when the temperature shift was initiated to 65.7 ± 8.3° six seconds post temperature shift). In contrast, lice did not appreciably change their angular displacement as temperatures mimicked movement towards flight feathers (36 → 32 °C) or during control trials (32 → 32 °C). Thus, after temperatures shift away from those found in their preferred bird regions, lice initiated large angular movements. This response is similar to the angular course control observed in the spatial heat gradient, where deviations from the ALB were followed by corrective returns towards the heat target.

Lice also decreased their velocity as the temperature shift mimicked movement towards flight feathers (36 → 32 °C), and increased velocity as temperatures mimicked movement away from flight feathers (32 → 36). Altering velocity in response to shifting temperatures was also seen for lice with unilateral antennectomies placed on spatial heat gradients, suggesting a similar control system was used in both circumstances.

4.3. Control systems

**Idiothetic control.** To test for the possibility of idiothetic control, we placed lice with unilateral antennectomies on heat gradients. These lice maintained bearings directed at the heat target while those with bilateral antennectomies reverted to a more random search behavior. This is consistent with lice using only temporal aspects of the thermal cue to guide movement. Course control likely occurred through corrective turns after the intensity of the cue changed, as seen by the correlation between the size of their deviation from the ALB and the size of subsequent returns towards the ALB.

Further support for idiothetic control comes from lice placed on temporal heat gradients. When temperatures shifted uniformly over time, mimicking a deviation away from a preferred temperature, lice showed a marked increase in the size of their angular movements consistent with corrective turning.

Finally, lice with unilateral antennectomies as well as those placed on the temporal heat gradient both altered velocity in response to changes in the stimulus intensity over time, consistent with idiothetic control.

Alternatively, orientation using a single antenna could be accomplished by comparing the stimulus between multiple thermal sensilla located across the antenna (as seen, for example, in Triatomine bugs; Flores & Lazzari 1996), or by using thermal sensilla located on other body regions. However, these are unlikely scenarios. Louse antennae are quite small (~300 μm) which would minimize any temperature differences between the base and distal tip of the antenna, and the ‘tuft’ organ thought to serve as a thermo-hygrosensilla has only been found on a single flagellum in louse antenna (Steinbrecht, 1994; Smith, 2001). Additionally, to our knowledge there have been no reports of thermal sensilla on louse body regions other than the antennae.

**Allothetic control.** It is likely that louse orientation on spatial heat gradients relied at least in part on detecting spatial aspects of the thermal cue. Lice with two antennae traveled straighter paths, took fewer and smaller turns, and located heat targets more effectively than those with unilateral antennectomies. Additionally, they did not alter velocity as the heat target was approached, suggesting their response relied more heavily on spatial rather than temporal aspects of the cue.

While it is not feasible to eliminate idiothetic control, there are circumstances when orientation can only be accomplished using the spatial component of the thermal cue. For instance, while lice traverse a temperature isocline (when their body-angle is perpendicular to the heat target), temperatures will remain stable over time. Thus, comparisons of the thermal cue over time would be of little use in orientation, and movement towards the heat target would only occur if lice responded to spatial differences in temperature between antennae.

When lice with two antennae were perpendicular to the heat target, their next angular movements were more often returns towards the heat target (64% of angular movements) rather than departures (36%). Furthermore, the average size of returns was significantly greater than that of departures, indicating lice are capable of detecting and responding to slight temperature differences between antennae. In contrast, lice with unilateral and bilateral antennectomies showed no ability to respond to spatial components of the thermal cue, performed a similar number of returns and departures, and showed no significant difference in the size of these returns and departures.

In all, our data show that lice possess the ability to use separate control systems to process spatial and temporal aspects of the thermal cue. We suggest that thermo-orientation likely results from the integration of both allothetic and idiothetic information.

4.4. Conclusions

The behavior of lice with unilateral antennectomies as well as those placed on temporal heat gradients indicates that directed movement on heat gradients can be accomplished using only temporal information (idiothetic control). Lice unable to detect spatial aspects of the thermal cue responded to changing temperatures by engaging in angular course control over time and by altering velocity. However, as lice with two antennae are capable of detecting spatial variations in temperature, it appears that allothetic control may also guide thermo-orientation. While it is beyond the scope of this study to determine which control mechanism is dominant, it is likely that lice integrate information from both control systems during thermo-orientation. Many factors are thought to impact the equilibrium between idiothetic and allothetic control, such as external inputs (e.g., wind, semiochemicals) and changes in internal states (e.g., body condition or feeding status; Lazzari, 2009). Further experiments are necessary to determine when and how both control systems are integrated during orientation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2019.103985.

References


