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A hitchhiker's guide to parasite transmission: The phoretic behaviour of feather lice

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ABSTRACT

Transmission to new hosts is a fundamental challenge for parasites. Some species meet this challenge by hitchhiking on other, more mobile parasite species, a behaviour known as phoresis. For example, feather-feeding lice that parasitise birds disperse to new hosts by hitchhiking on parasitic louse flies, which fly between individual birds. Oddly, however, some species of feather lice do not engage in phoresis. For example, although Rock Pigeon (*Columba livia*) "wing" lice (*Columbicola columbae*) frequently move to new hosts phoretically on louse flies (*Pseudolynchia canariensis*), Rock Pigeon "body" lice (*Campanulotes compar*) do not. This difference in phoretic behaviour is puzzling because the two species of lice have very similar life cycles and are equally dependent on transmission to new hosts. We conducted a series of experiments designed to compare the orientation, locomotion and attachment capabilities of these two species of lice, in relation to louse flies. We show that wing lice use fly activity as a cue in orientation and locomotion, whereas body lice do not. We also show that wing lice are more capable of remaining attached to active flies that are walking, grooming or flying. The superior phoretic ability of wing lice may be related to morphological adaptations for life on wing feathers, compared to body feathers.

1. Introduction

Transmission between hosts is one of the most important aspects of a parasite's life history. Because every host eventually dies, transmission to new host individuals is essential for the persistence of parasite lineages. Furthermore, transmission has profound ecological and evolutionary consequences for host–parasite interactions. Transmission influences parasite population dynamics, virulence (Ewald, 1994) and host specificity (Combes, 2001; Poulin, 2007). From an evolutionary perspective, transmission mediates gene flow (Criscione et al., 2005), thereby impacting upon local adaptation (Lajeunesse and Forbes, 2002) and host–parasite coevolution (Huyse et al., 2005; Johnson and Stinchcombe, 2007). While this large body of empirical and theoretical work speaks to the wide-ranging impacts of transmission, behavioural mechanisms underlying many modes of transmission are poorly understood.

Transmission can be particularly challenging for parasites that are highly specialised for life on the host. For example, feather lice (Phthiraptera: Ischnocera) are wingless, permanent parasites of birds that complete all stages of their life cycle on the host's body (Marshall, 1981). Because feather lice have poor mobility and survival off the host, transmission often occurs during periods of direct contact between hosts, like that between parents and offspring in the nest (Rothschild and Clay, 1952; Marshall, 1981). In some cases, however, feather lice have evolved alternative modes of transmission. One of the most interesting examples is phoretic hitchhiking on hippoboscid "louse flies" (Fig. 1), which are mobile, haematophagous parasites of birds that are distributed from the tropics to warmer temperate regions (Marshall, 1981). Louse flies spend most of their time in the plumage of birds, where their dorso-ventrally flattened bodies enable them to move efficiently between feathers. While they are relatively weak fliers, hippoboscid flies do leave the host to deposit pupae off the host and to fly between hosts (Marshall, 1981). Early work examining the prevalence of phoresis documented that 20–44% of individuals in some fly populations carried lice (Markov, 1938; Edwards, 1952; Corbet, 1956; Baum, 1968; Bennett, 1961). Keirans (1975a) reviewed hundreds of cases of feather louse phoresis, and found that 44% involved multiple lice on a fly, with up to 31 lice found on a single fly.

The ability to transmit phoretically may have important consequences for host–parasite interactions. Harbison et al. (2008) showed that phoresis can be an important mechanism for escaping competition with other species of lice on the same host. Additionally, phoresis may provide lice with a means of encountering novel host species, leading to a reduction in host specificity. Hippoboscid flies are usually less host specific than the lice they carry; many fly species infest multiple bird orders, while lice are typically restricted to a few host species or genera (Marshall, 1981; Price et al., 2003). Lice capable of phoresis are, therefore, expected to occur on a more diverse assemblage of host species than non-phoretic lice (Clayton et al., 2004; Harbison, 2008, Ecology and evolution of transmission in feather-feeding lice (Phthiraptera: Ischnocera). Ph.D. Thesis, University of Utah, USA).

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Fig. 1. Historical illustration of three feather lice hitchhiking a ride on the abdomen of a louse fly (from Rothschild and Clay, 1952).

Although phoresis is common among unrelated groups of feather lice, some taxa are seldom, if ever, phoretic (Keirans, 1975a). This difference in phoresis is puzzling because all lice are dependent on transmission to new hosts. Furthermore, because feather lice are essentially immobile off the host, they can all presumably benefit from the ability to "hitchhike" away from a dead or dying host. The overriding purpose of this study was to ask the question "why are some feather lice not phoretic"? Specifically, we tested the hypothesis that differences in phoresis may be related to biomechanical constraints. In short, some species of lice may simply not have the sensory or morphological "equipment" required for phoresis. A successful bout of phoresis requires that the hitchhiking species, or "phoront", must locate a potential carrier, move towards that carrier, attach to it and remain attached until the carrier reaches a new host individual. It is conceivable that differences in phoresis among lice could be due to differences in the ability to perform these tasks.

Parasites orient to hosts using chemical, visual, tactile or auditory cues (Rea and Irwin, 1994; Gibson and Torr, 1999; Owen and Mullens, 2004). Phoronts are known to use similar cues to orient to potential carriers (Binns, 1982; Niogret et al., 2006). For example, herbivorous broad mites (*Polyphagotarsoemus latus*) are attracted to semiochemicals on the cuticle of their whitefly carriers (Soroker et al., 2003). Blister beetles (*Meloe franciscanus*) essentially turn the tables by mimicking the sex-pheromone of female bees to lure their male bee carriers (Saul-Gershenz and Millar, 2006). Vision may also play a role in the remarkable case of the mite, *Hericia laboratorium*, which leaps 2–5 cm into the air to attach to flying insect carriers (Hall, 1959; Binns, 1982). One of the main goals of our study was to provide a preliminary assessment of what cues might play a role in the location of carriers by phoretic lice.

We used a model system consisting of Rock Pigeons (*Columba livia*) and their wing lice (*Columbicola columbae*) and body lice (*Campanulotes compar*). Although not closely related, these two species of lice display similar life histories and can be considered ecological "replicates" (Johnson and Clayton, 2003; Johnson et al., 2005). Both species cement their eggs to host feathers (Marshall, 1981), develop from eggs to adults in 3–4 weeks (Martin, 1934) and feed on the bird's abdominal contour feathers (Bush and Malenke, 2008). Despite these similarities, recent experiments show that wing lice are commonly phoretic on the hippoboscid fly, *Pseudolynchia canariensis*, while body lice are virtually never phoretic (Harbison et al., 2008). This difference occurs despite the fact that flies are found more frequently on regions of the bird where body lice are more abundant (Harbison et al., 2008).

We conducted a series of experiments to test whether wing and body lice detect active flies a few centimetres away and what cues they might be using in detection, orientation and movement towards flies. We also conducted a series of experiments to compare the ability of wing and body lice to attach to active flies and remain attached while the flies walked, groomed or flew. Our results show that wing lice orient to flies, probably using fly activity as a cue, and that, once attached, they are capable of remaining attached to active flies. Body lice, in contrast, show no interest in flies and, when "forced" to attach, they do not remain attached to moving flies nearly as well as do wing lice. These differences appear to be mediated by differences in behaviour related to morphological adaptations for life on wing feathers, compared to body feathers.

2. Materials and methods

2.1. Orientation and movement of lice

To test whether lice orient to, and move towards flies, we conducted a series of assays in which lice were placed in the centre of a toothpick "bridge", with a fly in a mesh enclosure at one end, and an empty enclosure at the other end (Fig. 2A–D). The 2.0 × 1.0 cm enclosures were made of 3 mm nylon mesh, allowing free circulation of air. The toothpick bridge was mounted 0.5 cm above the bottom of a glass Petri dish on a straight metal pin attached to a

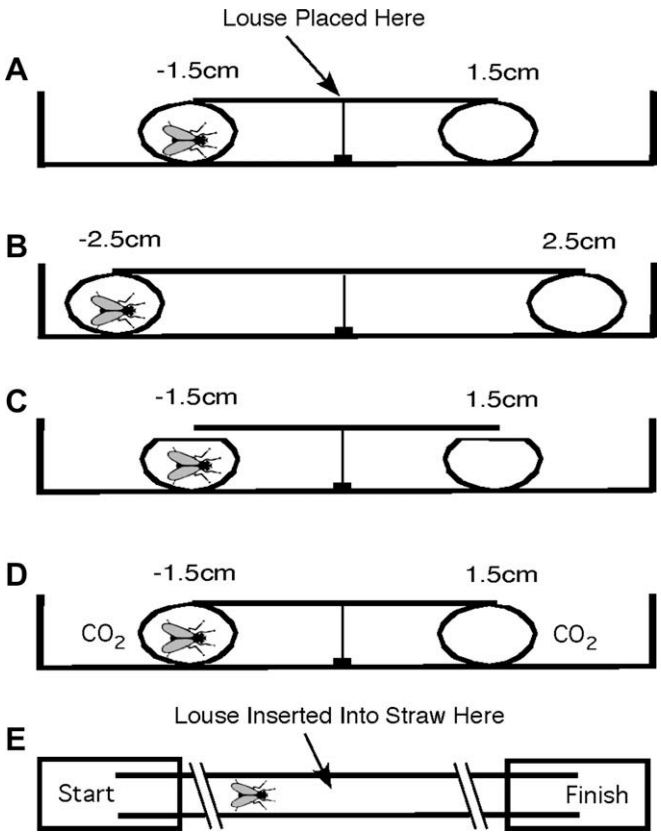

Fig. 2. Apparatus used for behavioural assays. Lice were placed at the centre of a toothpick "bridge", perpendicular to its long axis. From this position they could move toward the mesh enclosure containing a fly, or towards the empty enclosure at the other end of the bridge: (A) 3 cm long bridge with ends touching the enclosures; (B) 5 cm long bridge with ends touching the enclosures; (C) 3 cm long bridge with ends not touching the (flattened) enclosures; (D) 3 cm long bridge with ends touching the enclosures; fly anaesthetised with CO_2; (E) apparatus used to measure attachment of lice to active flies (10 cm long × 0.5 cm diameter soda straw with 4 cm long × 1 cm diameter glass vial at each end). Lice were inserted midway between the start and finish vials through a small hole in the straw.

wooden base. The bridge overlapped each enclosure by 0.5 cm. A ruler was placed beneath the dish for easy documentation of the distance travelled by lice from the midpoint (0 cm) of the bridge.

Each louse was placed on the bridge with jeweler's forceps. Lice normally moved within the first few seconds and we recorded whether they initially turned in the direction of the fly-containing enclosure, or in the direction of the empty enclosure. Next, we recorded the position of the louse on the bridge after 1 min, and again after 2 min. Both wing and body lice were capable of traversing the entire length of the bridge from one end to the other during the 2-min trial.

For each assay, we conducted trials for 30 wing lice and 30 body lice. Trials were carried out at room temperature in a small, isolated room with diffuse overhead lighting. A new louse was used for each trial and a new fly was used for every 10 trials. A new toothpick "bridge" was used for each trial and the Petri dish was washed and dried between trials. Toothpicks were tapered slightly, with the narrower end averaging 0.16 cm, and the wider end averaging 0.18 cm. Half of the trials had the fly enclosure at the narrow end, and half had it at the wide end. Flies and lice were obtained from culture stocks raised on captive Rock Pigeons kept in an animal facility at the University of Utah (Animal Care and Use Committee Protocol # 05-08009).

For the first two experiments, the ends of the bridge were in direct contact with the enclosures to allow any vibration from the fly to be transmitted along the length of the toothpick. The first experiment had a 3 cm long bridge (Fig. 2A); the second experiment had a 5 cm long bridge (Fig. 2B). Other aspects of these two experiments were identical.

In the third experiment, the mesh bags were flattened slightly so that the ends of the bridge did not contact the enclosures (Fig. 2C). This prevented vibration from the fly from being transmitted to the 3 cm toothpick. However, this manipulation did not change the overall position of the fly, relative to the bridge.

The fourth experiment was a repeat of the first experiment with a 3 cm long bridge, but used flies that were anaesthetised with CO_2 (Fig. 2D). In each trial, the fly was put in a mesh enclosure identical to the previous experiments. The enclosure was then placed in a CO_2 chamber for 7 min, which immobilised the fly for about 3 min. We placed the other (empty) mesh enclosure in a separate CO_2 chamber for an equal amount of time before arranging the two enclosures on the apparatus and initiating the 2-min trial by placing a louse on the bridge (Fig. 2D).

2.2. Attachment of lice to flies

We used the apparatus shown in Fig. 2E to compare the ability of wing and body lice to (i) attach to an active fly and (ii) remain attached to the fly as it walked a distance of 5 cm. After placing a fly in the start vial, a single louse was inserted into a small hole in the middle of the translucent straw. After a 2-min period to allow the fly and louse to become acclimated, we gently slid the straw over the fly, causing it to walk through the straw to the finish vial. Midway through the straw, as the fly came into contact with the louse, we recorded whether the louse attached to the fly. We then recorded whether the louse was still attached to the fly as it exited the straw into the finish vial. We also measured the amount of time it took the fly to walk through the straw. In total, 100 trials were conducted using wing lice, and 100 trials using body lice, alternating between the two species of lice. A new louse was used for each trial, and a new fly was used for every 20 trials.

Next, we compared the ability of wing and body lice to withstand grooming by flies. A fly was placed in a $10 \times 10 \times 18$ cm clear plastic observation chamber and allowed to acclimate for 1 min. A louse was then placed in the chamber, which was tilted gently in different directions until the louse contacted then grasped onto the fly. The observation period began when the louse first attached to the fly and ended when the louse fell off the fly, or after 10 min, whichever came first. We recorded the amount of time each fly spent grooming during the 10-min trial. We also recorded the location of lice on different body regions of the fly, and the apparent cause of detachment. We had three treatments: 30 trials using wing lice, 30 trials using body lice and 30 control trials with no lice. A different louse was used for each trial, and a different fly for every trial sequence, which included the three treatments in random order.

Finally, we compared the ability of wing and body lice to remain attached to flies allowed to fly about 3 m. We conducted our flight trials in a rectangular room ($3.5 \times 1.5 \times 3$ m) with a large window at one end. For each trial, a louse and a fly were confined in the end of a clear plastic straw by bending the straw slightly. The louse was allowed to crawl onto the restricted fly for 30–60 s until the louse gripped the fly and stopped moving. The straw was then straightened out so that the fly could launch itself and escape from the straw. Because louse flies are positively phototactic, they flew directly from the straw to the window 3 m away. Once it landed on the windowpane, a glass vial was placed over the fly, and the presence or absence of the louse noted. We then carefully searched the ledge beneath the window for lice that may have been dislodged from the fly when it contacted the window. We conducted 35 trials using wing lice and 35 trials using body lice, alternating between the two species for each trial. A new louse was used for each trial, and a new fly for every 10 trials. All trials were run during the same 3-h time period (over several days) to minimise differences in ambient lighting.

3. Results

3.1. Orientation and movement of lice

For our first experiment, we tested whether wing lice and body lice could orient and move towards flies using a 3 cm bridge that was in contact with a fly-containing mesh bag at one end, and an empty bag at the other end. Twenty-three of 30 wing lice (77%) turned towards the fly within the first few seconds of being placed on the bridge, rather than away from the fly ($\chi^2 = 8.53$, df = 1, $P < 0.01$). After initial orientation, wing lice tended to walk more or less continuously. While they occasionally reversed direction, they usually moved towards the end of the bridge with the enclosure containing the fly. At the end of the 2-min observation period, 23 wing lice were found on the half of the bridge near the fly, compared to six lice on the half with the empty enclosure (Fig. 3A; $\chi^2 = 9.97$, df = 1, $P < 0.005$). A single louse was found at the midpoint of the bridge at the end of the trial. Sixteen of the 30 wing lice actually moved off the bridge onto the fly enclosure, compared to just one wing louse that moved onto the empty enclosure ($\chi^2 = 13.23$, df = 1, $P < 0.01$).

In contrast, body lice showed no evidence of orienting to flies. After being placed on the bridge, 13 body lice initially turned towards the enclosure containing the fly, compared to 17 body lice that turned away from the fly enclosure ($\chi^2 = 0.53$, df = 1, $P = 0.47$, power = 0.78). Body lice often reversed direction on the bridge and showed no evidence of overall movement towards flies. Although body lice walked at a slower speed than wing lice, they too were capable of reaching either end of the bridge. After 2 min, 13 body lice were found on the 'fly half' of the bridge, compared to 12 on the half with the empty enclosure (Fig. 3C; $\chi^2 = 0.40$, df = 1, $P = 0.84$, power = 0.70). Many of the body lice were near the midpoint of the bridge at the end of trial, with five lice directly at the midpoint. After 2 min, a single body louse had moved onto the fly enclosure and a single body louse had moved onto the empty enclosure ($\chi^2 = 0.0$, df = 1, $P = 1.0$).

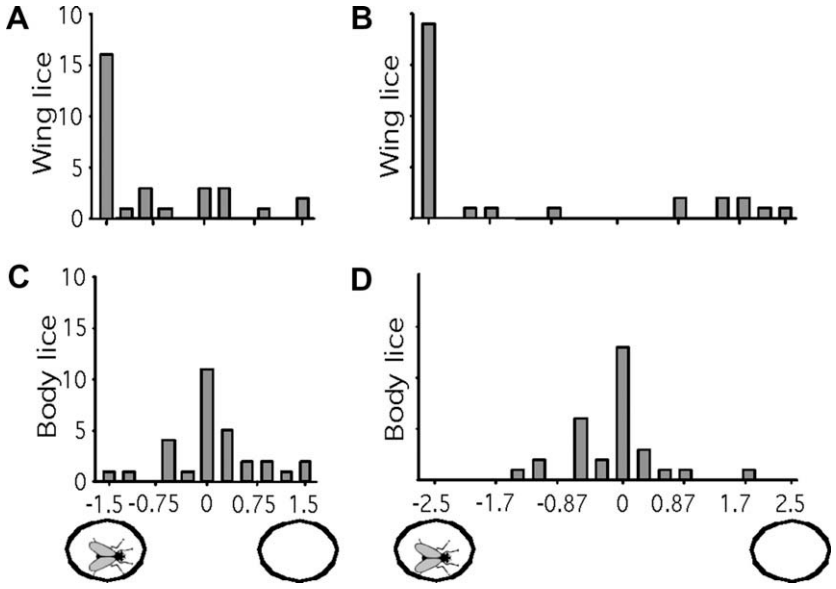

Fig. 3. Position (cm) of lice 2 min after being placed on bridge touching both enclosures (Fig. 2A and B). (A) Wing lice on 3 cm bridge; (B) wing lice on 5 cm bridge; (C) body lice on 3 cm bridge; (D) body lice on 5 cm bridge.

In our second experiment, we tested the ability of wing and body lice to detect flies from a greater distance. We repeated the methods from the first experiment, but extended the bridge from 3 to 5 cm. The results were similar. After 2 min, 22 wing lice were found on the half of the bridge near the fly, compared to eight wing lice found on the half near the empty enclosure (Fig. 3B; $\chi^2 = 6.53$, df = 1, $P = 0.01$). A total of 19 wing lice moved off the bridge onto the fly enclosure, compared to just one wing louse on the empty enclosure ($\chi^2 = 16.20$, df = 1, $P < 0.01$). As before, body lice showed no significant movement towards flies. After 2 min, 11 body lice were found on the 'fly half' of the bridge, compared to 13 lice on the half with the empty enclosure (Fig. 3D; $\chi^2 = 0.17$, df = 1, $P = 0.68$, power = 0.69). Many body lice again remained near the midpoint, with six lice directly at the midpoint. Unlike trials on the 3 cm bridge, body lice did not reach either end of the bridge after 2 min due to their slower speed.

In our third experiment, we slightly flattened both mesh bags, so they no longer contacted the 3 cm bridge. Wing lice still oriented to flies, although the intensity of their response was reduced, with 21 of 30 lice (70%) turning towards the fly ($\chi^2 = 4.80$, df = 1, $P = 0.03$). However, in contrast to the previous experiments, this initial orientation was not followed by significant movement in the direction of the fly. At the end of the 2 min trials, 14 lice were found on the 'fly half' of the bridge, compared to 13 on the half with the empty enclosure (Fig. 4A; $\chi^2 = 0.37$, df = 1, $P = 0.85$, power = 0.74); three lice remained directly at the midpoint. It was not possible for lice to move onto enclosures, since they were no longer in contact with the bridge.

Body lice showed a tendency to orient to flies (20 lice) rather than away from them (10 lice), but the difference was not significant ($\chi^2 = 3.33$, df = 1, $P = 0.07$, power = 0.78). After 2 min, 17 body lice were found on the 'fly half' of the bridge, compared to 10 on the half with the empty enclosure (Fig. 4B; $\chi^2 = 1.82$, df = 1, $P = 0.18$, power = 0.74); three lice remained directly at the midpoint.

In our fourth experiment, we tested whether wing lice were capable of orienting and moving towards flies that were anaesthetised with CO_2. Wing lice no longer oriented to flies. After being placed on the bridge, 16 wing lice turned towards the fly, compared to 14 lice that turned away ($\chi^2 = 0.13$, df = 1, $P = 0.72$, power = 0.78). Wing lice also showed no evidence of movement towards immobilised flies. After 2 min, 11 wing lice were found on the 'fly half' of the bridge, compared to 15 lice on the half with the empty enclosure (Fig. 5; $\chi^2 = 0.62$, df = 1, $P = 0.43$, power = 0.72); four lice were directly at the midpoint. Four wing lice had moved onto the mesh enclosure containing the immobilised fly, and four had moved onto the empty enclosure ($\chi^2 = 0.0$, df = 1, $P = 1.0$, power = 0.29). We did not test responses of body lice to anaesthetised flies.

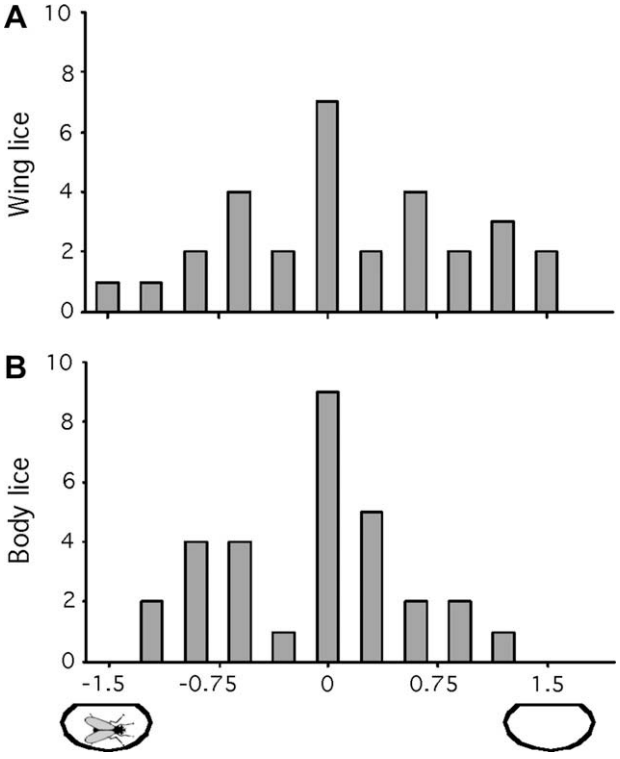

Fig. 4. Position (cm) of lice 2 min after being placed on bridge not touching either enclosure (Fig. 2C). (A) Wing lice on 3 cm bridge; (B) body lice on 3 cm bridge.

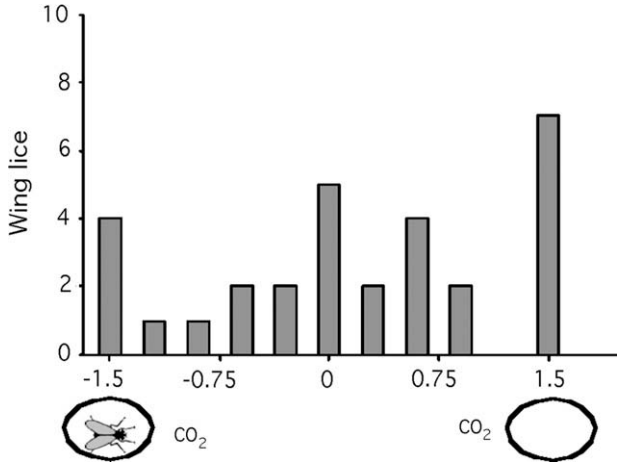

Fig. 5. Position (cm) of wing lice 2 min after being placed on bridge touching both enclosures; fly anaesthetised with CO_2 (Fig. 2D).

3.2. Attachment of lice to flies

Our first attachment experiment compared the ability of wing lice and body lice to first attach to a moving fly, and then remain attached while the fly walked 5 cm. Thirty-five of 100 wing lice attached to moving flies, compared to 32 of 100 body lice ($\chi^2 = 0.20$, df = 1, $P = 0.65$, power = 1.0). Both kinds of lice attached to the legs of flies, although the precise attachment mechanism could not always be observed, given the speed of the interaction and small size of the lice. Possible mechanisms included lice using their tarsal claws to grasp the setae (hairs) of the fly, lice wrapping their legs around narrow regions of the fly's legs or lice grasping fly setae with their mandibles. Each of these mechanisms was observed during pilot trials in which we placed lice directly onto flies and observed them under magnification.

Although wing and body lice did not differ in rates of attachment to flies, about a third of all body lice detached from flies as they walked through the straw, whereas only two wing lice detached (Fig. 6; Fisher's Exact, $n = 67$ lice, $P < 0.005$). The mean (±95% Confidence Interval (CI)) time it took flies with wing lice to travel 5 cm was 17.63 s (12.76–27.33) compared to 27.41 s (21.27–33.54) for flies with body lice, a non-significant difference (two-tailed Wilcoxon Rank-Sums, $n = 67$, $z = 1.57$, $P = 0.11$).

Next, we compared the ability of wing and body lice to withstand fly grooming. Flies spent a mean (±95% CI) of 15.5% (8.6–22.5) of their time grooming during wing louse trials, 13.9% (6.3–21.4) of their time grooming during body louse trials, and 17.0% (8.7–25.4) of their time grooming in control trials. The percentage of time flies groomed was not significantly different between wing louse, body louse and control trials (two-tailed Wilcoxon Rank-Sums, $\chi^2 = 1.24$, df = 2, $P = 0.54$). However, wing lice were better able to withstand fly grooming than body lice: fewer wing lice were groomed off compared to body lice (Fig. 6; Fisher's Exact, $n = 60$, $P < 0.005$). Ten wing lice remained attached to flies for the entire 10-min observation period, compared to just one body louse. Indeed, several wing lice remained attached well beyond the observation period, with one individual remaining attached to a fly for 6 h.

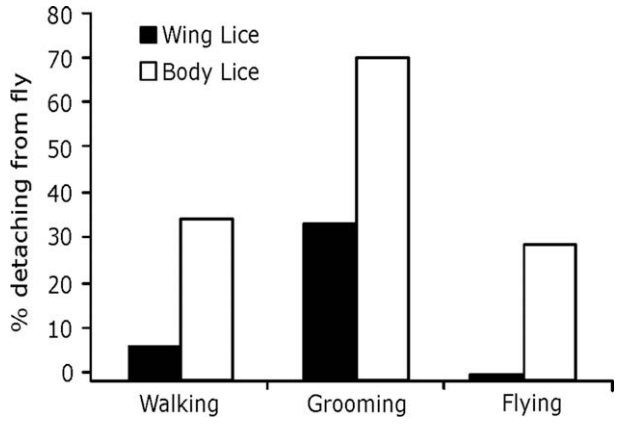

Fig. 6. Comparison of detachment rates by wing and body lice from flies that were walking (5 cm), grooming (10 min) or flying (3 m).

Finally, we compared the ability of wing and body lice to remain attached to a fly during flight. Immediately prior to flight, a majority of wing lice (71%) and body lice (66%) had attached to the back legs of flies in the bent straw; other lice attached to the middle legs (wing lice, 11%; body lice, 17%), front legs (wing lice, 14%; body lice, 11%) and abdomen (wing lice, 3%; body lice, 6%). There was no significant difference in the number of wing versus body lice attached to these four regions prior to flight ($\chi^2 = 0.93$, df = 3, $n = 70$, $P = 0.82$). However, wing lice were significantly better at remaining attached to flying flies: 0% of wing lice fell off during the 3 m flight, compared to 29% of body lice (Fig. 6, Fisher's Exact, $n = 70$, $P < 0.005$).

4. Discussion

Phoresis is a widespread behaviour amongst arthropods that allows immobile taxa to locate patchy or ephemeral resources, including hosts (Farish and Axtell, 1971; Binns, 1982; Brown and Wilson, 1992; Saul-Gershenz and Millar, 2006). We tested whether differences in phoresis by Rock Pigeon wing and body lice are due to differences in the underlying behavioural components required for phoresis. Our results show that: (i) wing lice orient and move towards active flies, whereas body lice show neither of these responses; (ii) wing lice continue to orient to active flies no longer in contact with the bridge, although the lice do not consistently move closer to such flies; (iii) wing lice do not respond to anaesthetised flies; (iv) wing lice and body lice attach to active flies at similar rates when forced into contact and (v) wing lice are much better than body lice at remaining attached to flies that are walking, grooming or flying. In summary, wing lice exhibit a variety of proximal behaviours consistent with phoresis, whereas body lice do not exhibit these behaviours.

To initiate phoresis, a louse must first orient to and move towards a potential carrier. Wing lice immediately oriented to, and then moved towards flies (Fig. 3A). They even crawled off the bridge and onto the mesh directly above flies. Similar results were found when the bridge was extended to 5 cm in length (Fig. 3B). Wing lice are only about 2 mm long; hence, our results show that they are able to detect flies at least 150 body lengths away, equivalent to a 2 m tall man orienting to a stimulus three football fields away. In contrast, body lice did not show any significant response to flies (Fig. 3C and D). Body lice typically took more time to choose a direction of initial movement, and they moved less continuously than wing lice.

Parasitic insects are known to orient to, and move towards, hosts using cues such as semiochemicals or host activity, including auditory and tactile cues (Combes, 1991; Rea and Irwin, 1994; Soroker et al., 2003; Owen and Mullens, 2004; Fatouros et al., 2005). Visual cues are unlikely in the case of lice, which have rudimentary vision, at best (Price et al., 2003). We ran two additional choice assay experiments in which the apparatus was modified to reduce, then eliminate, fly activity as a potential cue used by lice. Flies were active during the trials, continuously walking, running, grooming or vibrating their wings in the enclosures. We first

eliminated contact between the bridge and the enclosures, preventing the transfer of tactile vibrations from the fly to the bridge. Wing lice still oriented to flies, suggesting that airborne vibrations (i.e. sound) or volatile semiochemicals might still be detected by the lice.

Although the wing lice continued to orient to flies no longer in contact with the bridge, they no longer accumulated at the fly end of the bridge (Fig. 4A). This may simply have been due to the inability of wing lice to exit the bridge onto the mesh enclosure. Because lice that initially moved towards the fly were ultimately unable to reach it, they may have switched into a random search behaviour, resulting in the normal distribution of lice across the bridge (Fig. 4A). Wing lice observed reaching the end of the bridge near the fly often reversed direction and moved away from the fly. Alternatively, wing lice may simply have modulated their behaviour based on the diminished intensity of cues. In this scenario, blocking tactile cues might have reduced, yet not eliminated, the response of wing lice to flies.

Wing lice showed no initial orientation nor movement towards flies anaesthetised with CO_2 (Fig. 5), suggesting that they do, in fact, cue into some aspect of fly activity, rather than semiochemicals. However, our experiments are not conclusive because it is possible that, like fly activity, semiochemicals were affected by CO_2. Additional choice assays using fly extracts are needed to clarify the role of volatile semiochemicals in the location of flies by lice.

It is possible that wing lice display a generalised response to cues that are not specific to flies. Lice have occasionally been reported riding on insects other than hippoboscid flies, including fleas, flies, dragonflies, bees, butterflies and mosquitoes (Keirans, 1975b). Other phoronts, such as mites, are known to use multiple carriers as well (Binns, 1982). Perhaps a generalised response to local activity explains wing louse phoresis on other carriers. Wing lice may even be attracted to tactile or auditory vibrations regardless of the presence of another insect. It would be interesting to repeat our experiments using mechanical agitation rather than actual hippoboscid flies.

Alternatively, by conducting the experiments off the host, one could argue that body lice did not respond to flies because they locate flies using cues that could not be detected using our experimental design. However, we presented lice with flies in a variety of contexts and at different distances, yet saw no measurable response. Additionally, wing lice, which have similar ecology, displayed an immediate and strong response to flies. Furthermore, the behavioural response of wing and body lice is consistent with the many published records of phoresis in Rock Pigeon lice, all of which involve wing lice; none involve body lice (Martin, 1934; Hathaway, 1943; Ansari, 1947; Ward, 1953; Iannacone, 1992; Clayton et al., 2004; Macchioni et al., 2005; Harbison et al., 2008).

Once a louse locates and moves towards a fly, it must attach to it and remain attached while the fly walks, attempts to groom the louse off its body and flies to a new host. While the percentage of time flies groomed did not differ between trials with and without lice, flies were often observed directly grooming body regions where lice were attached (Harbison, personal observation). Wing and body lice attached to flies at similar rates, suggesting that body lice are capable of initiating a phoretic event. Once on flies, however, body lice detached at all stages of phoresis significantly more often than did wing lice (Fig. 6).

The difference in wing and body louse attachment to flies may result from morphological specialisations to different microhabitat regions on the host. Wing lice spend the majority of their time on the flight feathers (wings and tail), moving onto the central body to feed (Clayton, 1991; Bush and Malenke, 2008). On the coarse flight feathers wing lice must withstand aerodynamic forces associated with beating wings and high airspeed. To remain attached to the bird, wing lice have long "outrigger" legs extending laterally from their bodies, giving them a wide stance. While on flies, wing lice typically adopt a similar posture and often extend their long legs out laterally to clasp fly setae, or completely wrap their legs around narrow regions of the fly's legs (Harbison, personal observation).

In contrast to wing lice, body lice spend all their time in a three-dimensional downy feather-matrix on the central body of the bird (Clayton, 1991). They have short legs that extend more directly beneath their bodies that may limit their ability to remain attached to flies. In short, specialisation to life in central body feathers may constrain the evolution of phoresis in body lice.

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References

Ansari, M.A.R., 1947. Associations between the Mallophaga and the Hippoboscidae infesting birds. J. Bombay Nat. Hist. Soc. 46, 509–516.
Baum, V.H., 1968. Biologie und Okologie der Amselfederlause. Angew. Parasitol. 9, 129–176.
Bennett, G.F., 1961. On three species of Hippoboscidae (Diptera) on birds in Ontario. Can. J. Zool. 39, 379–406.
Binns, E.S., 1982. Phoresy as migration – some functional aspects of phoresy in mites. Biol. Rev. 57, 571–620.
Brown, J.M., Wilson, D.S., 1992. Local specialization of phoretic mites on sympatric carrion beetle hosts. Ecology 73, 463–478.
Bush, S.E., Malenke, J.R., 2008. Host defense mediates interspecific competition in ectoparasites. J. Anim. Ecol. 77, 558–564.
Clayton, D.H., 1991. Coevolution of avian grooming and ectoparasite avoidance. In: Loye, J.E., Zuk, M. (Eds.), Bird–Parasite Interactions: Ecology, Evolution, and Behaviour, Oxford Ornithology Series. Oxford University Press, Oxford, pp. 258–289.
Clayton, D.H., Bush, S.E., Johnson, K.P., 2004. Ecology of congruence: Past meets present. Syst. Biol. 53, 165–173.
Combes, C., 1991. Ethological aspects of parasite transmission. Am. Nat. 138, 867–880.
Combes, C., 2001. Parasitism: The Ecology and Evolution of Intimate Interactions. University of Chicago Press, Chicago and London.
Corbet, G.B., 1956. The phoresy of Mallophaga on a population of *Ornithomyia fringillina* Curtis (Dipt , Hippoboscidae). Entomol. Mon. Mag. 92, 207–211.
Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Mol. Ecol. 14, 2247–2257.
Edwards, R., 1952. Flatflies taken in the laboratory during 1951. Fair Isle Bird Obs. Bull. 6, 37–38.
Ewald, P.W., 1994. Evolution of Infectious Disease. Oxford University Press.
Farish, D.J., Axtell, R.C., 1971. Phoresy redefined and examined in *Macrocheles muscaedomesticae* (Acarina: Macrochelidae). Acarology 13, 16–29.
Fatouros, N.E., Huigens, M.E., Loon, J.J.A., Dicke, M., Hilker, M., 2005. Butterfly anti-aphrodisiac lures parasitic wasps. Nature 433, 704.
Gibson, G., Torr, S.J., 1999. Visual and olfactory responses of haematophagous Diptera to host stimuli. Med. Vet. Entomol. 13, 2–23.
Hall, C.C., 1959. A dispersal mechanism in mites. J. Kans. Entomol. Soc. 32, 45–46.
Harbison, C.W., Bush, S.E., Malenke, J., Clayton, D.H., 2008. Comparative transmission dynamics of competing parasite species. Ecology 89, 3186–3194.
Hathaway, C.R., 1943. Associacao entre Mallophaga e Hippoboscidae. Mem. Inst. Oswaldo Cruz 38, 413–417.
Huyse, T., Poulin, R., Théron, A., 2005. Speciation in parasites: a population genetics approach. Trends Parasitol. 21, 469–475.
Iannacone, J.A., 1992. Registro de un caso de phoresis: *Columbicola columbae* (L.) (Phthiraptera: Insecta) por *Pseudolynchia canariensis* (Diptera: Insecta) en la zona de Lima. Peru Bol. Lima 84, 17–18.
Johnson, K.P., Clayton, D.H., 2003. Coevolutionary history of ecological replicates: comparing phylogenies of wing and body lice to Columbiform hosts. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogenies, Cospeciation, and Coevolution. The University of Chicago Press, Chicago and London, pp. 262–285.
Johnson, K.P., Bush, S.E., Clayton, D.H., 2005. Correlated evolution of host and parasite body size: tests of Harrison's rule using birds and lice. Evolution 59, 1744–1753.
Johnson, M.T.J., Stinchcombe, J.R., 2007. An emerging synthesis between community ecology and evolutionary biology. Trends Ecol. Evol. 22, 250–257.

Keirans, J.E., 1975a. A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). J. Med. Entomol. 12, 71–76.
Keirans, J.E., 1975b. Records of phoretic attachment of Mallophaga (Insecta: Phthiraptera) on insects other than Hippoboscidae. J. Med. Entomol. 12, 476.
Lajeunesse, M.J., Forbes, M.R., 2002. Host range and local parasite adaptation. Proc. R. Soc. Lond. B Biol. Sci. 269, 703–710.
Macchioni, F., Magi, M., Mancianti, F., Perruci, S., 2005. Phoretic association of mites and Mallophaga with the pigeon fly *Pseudolynchia canariensis*. Parasite 12, 277–279.
Markov, G.S., 1938. The presence of phoresy in Mallophaga. Zool. Zh. 17, 634–636.
Marshall, A.G.., 1981. The Ecology of Ectoparasitic Insects. Academic Press, London.
Martin, M., 1934. Life history and habits of the pigeon louse (*Columbicola columbae* [Linnaeus]). Can. Entomol. 66, 6–16.
Niogret, J., Lumaret, J.P., Bertrand, M., 2006. Semiochemicals mediating host-finding behaviour in the phoretic association between *Macrocheles saceri* (Acari: Mesostigmata) and *Scarabaeus* species (Coleoptera: Scarabaeidae). Chemoecology 16, 129–134.
Owen, J.P., Mullens, B.A., 2004. Influence of heat and vibration on the movement of the Northern Fowl Mite (Acari: Macronyssidae). J. Med. Entomol. 41, 865–872.
Poulin, R., 2007. Evolutionary Ecology of Parasites. Princeton University Press, Princeton.
Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H., 2003. The chewing lice world checklist and biological overview. Illinois Natural History Survey Special Publication 24.
Rea, J.G., Irwin, S.W.B., 1994. The ecology of host finding behavior and parasite transmission: past and future perspectives. Parasitology 109, s31–s39.
Rothschild, M., Clay, T., 1952. Fleas, Flukes, and Cuckoos. Collins, London.
Saul-Gershenz, L.S., Millar, J.G., 2006. Phoretic nest parasites use sexual deception to obtain transport to their host's nest. Proc. Natl. Acad. Sci. USA 103, 14039–14044.
Soroker, V., Nelson, D.R., Bahar, O., Reneh, S., Yablonski, S., Palevsky, E., 2003. Whitefly wax as a cue for phoresy in the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae). Chemoecology 13, 163–166.
Ward, R.A., 1953. Additional record of phoresy of Mallophaga on Hippoboscidae. Bull. Brooklyn Entomol. Soc. 48, 128.