Does behavioural flexibility facilitate host switching by parasites?

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Summary

1. Baldwin (1896) suggested that behavioural flexibility could allow organisms to persist in novel environments, thus buying time for the evolution of genetic adaptations to the new environment. This has proven true for free-living organisms invading novel habitats. Behavioural flexibility could also allow parasites to exploit novel hosts, but this hypothesis has not been tested, despite the fact that parasitism is one of the most common lifestyles on earth.

2. In this study, I compare the behavioural flexibility of two relatively host specific groups of feather lice (Phthiraptera: Ischnocera) that parasitize pigeons and doves: 'wing' lice (*Columbicola columbae* and *C. passerinae*) and 'body' lice (*Campanulotes compar* and *Physconelloides eurysema*).

3. Wing and body lice are similar in many aspects of their natural history, but they differ in their relationship to host body size. Wing louse size is tightly correlated with host body size, whereas body louse size is not. Even so, experiments have shown that wing lice can establish on different sized novel hosts just as well as body lice.

4. Behavioural flexibility may facilitate the establishment of wing lice on different sized novel hosts. To test this hypothesis, I experimentally transferred wing and body lice to a series of different sized novel host species. Once the louse populations established (two generations) I compared the microhabitat preferences of wing and body lice on novel hosts vs. native host controls.

5. Wing lice shifted their microhabitat use on novel host species, and the magnitude of the shift was correlated with host size. In contrast, body lice rarely shifted microhabitat and when they did, the shift was not correlated with host size.

6. Behavioural flexibility may play a pivotal role in the ability of wing lice to establish on different sized novel host species, and could be an important factor for other parasites faced with novel hosts.

Key-words: behavioural plasticity, host-specificity, pigeons, doves, lice, Harrison's rule, Phthiraptera, Ischnocera

A shift into a new niche or adaptive zone is almost without exception initiated by a change in behavior.

Mayr (1963)

Introduction

Parasitism is one of the most common lifestyles on Earth (Price 1980). Although many parasites are relatively host-specific, factors governing the evolution of specificity remain poorly understood (Poulin 2007; Poulin & Keeney 2008). One factor that likely contributes to host-specificity is the tendency of parasites to be locally adapted to the environment provided by a particular host species (Lajeunesse & Forbes 2002).

Morphological, behavioural or physiological characteristics that allow a parasite to efficiently exploit one host can make it difficult for the parasite to exploit other hosts. Adaptation to a particular host may explain why sympatric host species sometimes do not share parasites, despite ample opportunities for dispersal (Joshi & Thompson 1995). In contrast, parasites do occasionally switch to new and even distantly related host species (Poulin & Mouillot 2003). Understanding factors that reinforce host-specificity, vs. those allowing parasites to establish on new host species, is central to understanding the evolution of parasite biodiversity.

Phenotypic plasticity can facilitate the establishment of organisms in novel environments (Yeh & Price 2004; Ghalambor *et al.* 2007). Behavioural flexibility, which is one type of phenotypic plasticity, may allow species to quickly cope with new environmental conditions. Baldwin (1896) proposed that behaviourally flexible species may be able to persist long

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enough in a novel environment to accumulate genetic changes and become locally adapted to that novel environment (Baldwin 1896). The 'Baldwin Effect' (Robinson & Dukas 1999) explains why the dark-eyed Junco (*Junco hyemalis*), which is typically a mountainous species, was able to invade and persist in a costal urban habitat (Yeh & Price 2004). Urban juncos exhibited flexibility in the timing and duration of the breeding season that allowed the birds to cope with new environmental pressures. Likewise, red fire ants (*Solenopsis invicta*) and Argentine ants (*Linepithema humile*) have undergone behavioural changes that make them more competitive in their introduced ranges (Holway & Suarez 1999). This behavioural plasticity is considered to be responsible, in part, for the success of these species in invading novel habitats.

Behavioural plasticity could conceivably facilitate host switching in parasites. Parasites that switch to novel hosts species are likely to experience an environment different from that of their native host. Behaviourally flexible parasites may be able to exploit new environments by using different micro-habitats on the novel hosts than they used on the native host. Although parasite specialization on particular host micro-habitats is considered to play a major role in the evolution of host-specificity (Rohde 1994), little is known about the constraints microhabitat specialization actually impose on parasites exposed to novel hosts. The potential role of micro-habitat shifts in host-switching was suggested by a short-term study of host-specific lice found on cave-swiftlets, in which lice experimentally transferred to a novel host species shifted their microhabitat distribution to feathers that were similar in size to those on the native host (Tompkins & Clayton 1999). However, the short (10 days) duration of the study did not allow the authors to test whether behavioural flexibility could lead to long-term establishment, including the reproduction of lice on novel host species. Experimental studies are needed to determine the role of behavioural flexibility in shaping ecological patterns of host-specificity and macroevolutionary patterns of host use and host switching by parasites.

In this paper, I explore the relationship between host-specificity, microhabitat specificity and behavioural flexibility in an experimentally tractable host–parasite system. I use feather lice on Columbiform birds (pigeons and doves) to test whether flexibility in microhabitat choice facilitates the establishment of parasites on novel hosts. I compare the microhabitat distributions of two groups of feather lice (Phthiraptera: Ischnocera) that co-occur on Columbiform birds: 'wing' lice in the genus *Columbicola*; and 'body' lice in the subfamily Physconelloidinae.

Columbiform wing and body lice are ecological 'replicates' (Clayton & Johnson 2003) that provide a powerful comparative context for testing the role of behavioural flexibility in host-switching. Both groups of lice are permanent ectoparasites that complete their entire life cycle on the body of the host (Marshall 1981). The 3–4 week life cycle begins with the egg, which is glued to the feathers, then progresses through three nymphal instars, to the adult stage. Wing and body lice move adeptly on the feathers and can travel about 4 cm min^{-1} (Bush 2004). These lice are so specialized for life on feathers that

they do not even venture onto the host's skin (Clayton 1991). Both groups feed on feathers, which are metabolized with the aid of endosymbiotic bacteria (Fukatsu *et al.* 2007). Transmission of lice to new hosts occurs mainly during periods of direct contact, like that between parents and their offspring in the nest. Most pigeon and dove species have both wing and body lice (Price *et al.* 2003a). Both groups of lice are relatively host-specific and exhibit significant phylogenetic congruence with their hosts, worldwide, revealing a long history of parasite–host association (Clayton & Johnson 2003).

The size of wing lice is correlated with host body size, a pattern known as Harrison's Rule (Johnson *et al.* 2005). Harrison's Rule is common among a diverse assemblage of parasites, including parasitic worms, crustaceans, fleas, flies, lice and ticks, as well as in herbivorous aphids, thrips, beetles, flies, moths and flower mites (Harvey & Keymer 1991; Kirk 1991; Thompson 1994; Poulin & Hamilton 1997; Sasal *et al.* 1999; Morand *et al.* 2000). The correlations between parasite and host size are likely driven by interactions that the parasites have with particular host features that scale with host size. For example, monogenean ectoparasites of fish attach to the gills of their hosts with hooks. Monogenean size is correlated with hook size, hook size is correlated with gill size, and gill size is correlated with overall fish size (Simková *et al.* 2001). Similarly, gopher lice hang onto individual host hairs with grooves on their heads (Reed, Hafner & Allen 2000). Rostral groove size is correlated with hair diameter, which is, in turn, correlated with overall host size. In the dove-louse system, wing louse size is correlated with feather size, which is correlated with overall host size. In contrast, the size of body lice does not correlate with feather size or host size (Johnson *et al.* 2005).

Given the adherence of wing lice to Harrison's Rule, one might predict that wing lice are more host-specific than body lice. Paradoxically, the reverse is true; wing lice are actually less host-specific than body lice (Johnson *et al.* 2002; Price *et al.* 2003a). Furthermore, experimental transfers by Bush & Clayton (2006) showed that both wing lice and body lice are able to establish viable populations on novel host species that vary in size by nearly an order of magnitude. Here I test the Baldwin Effect prediction that behavioural flexibility facilitates the establishment of wing lice on different sized novel hosts. Wing and body lice were experimentally transferred from a large-bodied native host species to progressively smaller hosts. In a reciprocal experiment, wing and body lice were also transferred from a small-bodied native host species to progressively larger hosts. I determined the behavioural flexibility of these lice by quantifying and comparing the microhabitat distributions of established louse populations on novel hosts and native host controls after two louse generations.

Methods

I collected data on microhabitat use by lice transferred experimentally among several species of captive Columbiformes (Fig. 1). All birds were wild individuals captured with nets or walk-in traps at the

Fig. 1. Design of parasite transfer experiments. Host species (drawn to scale) varied in size by more than an order of magnitude (body masses from Bush & Clayton 2006): C.G-D. = common ground-dove (*Columbina passerina*, 45 g); Z.D. = zebra dove (*Geopelia striata*, 50 g); M.D. = mourning dove (*Zenaida macroura*, 113 g); W-t.D. = white-tipped dove (*Leptotila verreauxi*, 177 g); B-t.P. = band-tailed pigeon (*Patagioenas fasciata*, 353 g); R.P. = rock pigeon (*Columba livia*, 364 g). In the first experiment (top arrows) lice from rock pigeons were transferred to progressively smaller novel host species, and to rock pigeon controls. In the second experiment (bottom arrows) lice from common ground-doves were transferred to progressively larger novel host species, and to common ground-dove controls. The host phylogeny is shown in grey (Clayton *et al.* 2003b; K. P. Johnson, unpub. data). In the first experiment, decreasing host size is correlated with increasing phylogenetic distance; the native host (R.P.) shares four common ancestors (nodes a–d) with the similar sized B-t.P., but only three common ancestors (a–c) with the smaller W-t.D., and so on. In contrast, increasing host size in the second experiment is independent of host phylogenetic distance because the native host (C.G-d.) shares just one common ancestor (a) with all four novel host species. Figure reprinted from Bush & Clayton (2006).
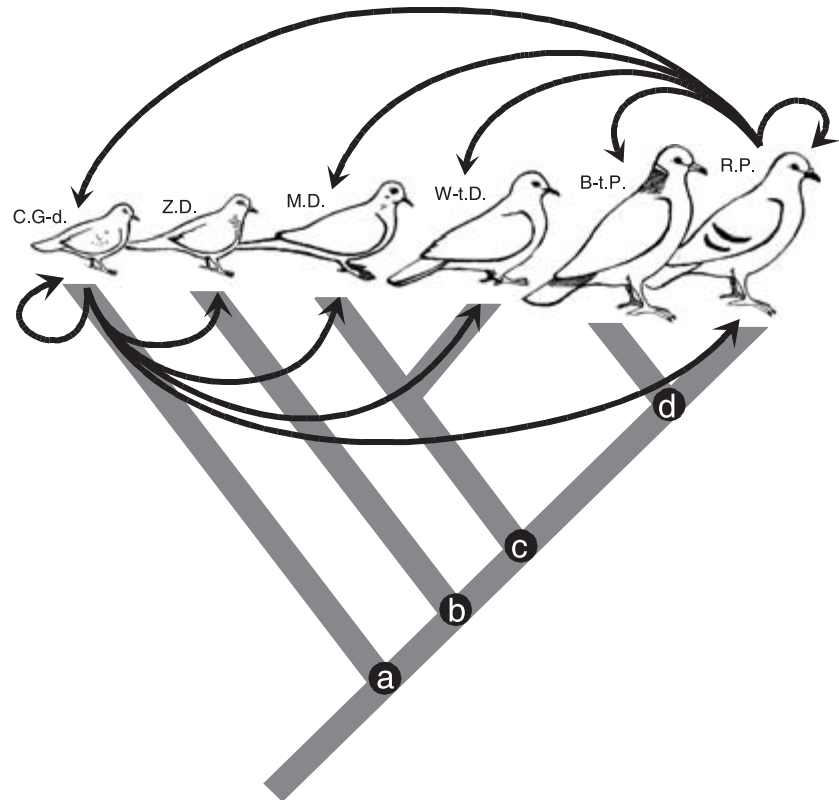
following localities: common ground-doves and white-tipped doves, Hidalgo Co. and Cameron Co. TX; zebra doves, Honolulu Co. HI; mourning doves, Pima Co. AZ; band-tailed pigeons, Benton Co. OR; rock pigeons, Salt Lake Co. UT. Captive birds were housed individually in $30 \times 30 \times 56$ cm wire mesh cages in an animal facility at the University of Utah. Cages were separated with plexi-glass partitions to prevent contact between the feathers of birds in adjacent cages, which could allow lice to move between birds. The birds were maintained on a 12-h light : dark photoperiod and provided *ad libitum* grain, grit and water.

Upon capture, natural louse infestations were exterminated by housing birds at < 25% relative humidity for ≥ 10 weeks following capture. This method kills lice and eggs, while avoiding residues that might result from the use of chemical fumigants (Harbison *et al.* 2008). Once natural louse infestations were exterminated, the relative humidity in the animal rooms was elevated to an optimum for lice (60%–70%) for the duration of this experiment (Nelson & Murray 1971; Bush & Clayton 2006). Next, each bird was seeded with lice from a culture stock bred on wild caught birds. The lice were removed from culture birds by anesthetizing them with CO_2 in a fumigation chamber (Clayton & Drown 2001; Moyer *et al.* 2002). They were then randomly divided into treatments and placed on recipient birds, which were restrained for an hour to allow lice to position themselves on the new host before returning it to a cage. To quantify microhabitat use by lice on novel hosts, all birds were visually examined after 2 months, as described below, which is approximately two louse generations (Martin 1934). This experimental design made it possible to determine if lice actually established viable, breeding populations on the novel hosts, and whether behavioural flexibility was central to their ability to establish.

TRANSFERS OF PIGEON LICE TO SMALLER NOVEL HOSTS

In the first experiment, I transferred wing lice (*Columbicola columbae*) and body lice (*Campanulotes compar*) from rock pigeons to four other Columbiform species that occur sympatrically in southern Texas (del Hoyo, Elliot & Sargatal 1997). Neither *C. columbae* nor *C. compar* occur on the four novel host species in the wild (Price *et al.* 2003a). The rock pigeon is the largest Columbiform species in the study; thus, in this experiment lice were transferred from the largest host species to four progressively smaller novel host species (Fig. 1, top arrows; *n* = 5–12 birds per species). The largest of the four novel hosts, the band-tailed pigeon, is similar in size to the rock pigeon (< 5% difference in body mass). It served as a positive control for comparing the microhabitat distribution of rock pigeon lice transferred to a similar sized novel host. Each bird received 25 adult wing lice and 25 adult body lice, which is roughly the equilibrium population size on captive rock pigeons with normal preening ability (Clayton *et al.* 2003, 2005).

TRANSFERS OF GROUND-DOVE LICE TO LARGER NOVEL HOSTS

In the second experiment, I transferred wing lice (*C. passerinae*) and body lice (*Physconelloides eurysema*) from the smallest host species, the common ground-dove, to four progressively larger hosts (Fig. 1, bottom arrows). Neither *C. passerinae* nor *P. eurysema* occur on the four novel hosts species in the wild. All of the species except the zebra dove occur sympatrically in southern Texas. The zebra dove is similar in size to the common ground-dove (< 10% difference in
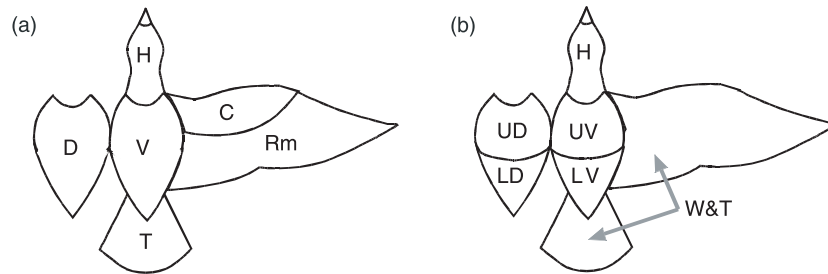
Fig. 2. Microhabitats for wing lice (a), and body lice (b). For wing lice the microhabitats were: (H) head, which included the crown, nape, gular and neck regions; (C) underwing coverts; (Rm) remiges, which are the flight feathers of the wing; (T) tail; (D) dorsal abdomen, which included the back, anterior rump and posterior rump; and (V) ventral abdomen, which included the breast, keel, side, flank and vent. For body lice the microhabitats were: (H) head as defined above, (W&T) wings and tail, which included the remiges, underwing coverts and tail; (UD) upper dorsal abdomen, which is the back; (LD) lower dorsal abdomen, which included the anterior and posterior rump; (UV) upper ventral abdomen, which is the breast; and (LV) lower ventral abdomen, which included the keel, side, flank and vent.

body mass). It served as a positive control for comparing the microhabitat distribution of common ground-dove lice transferred to a similar sized novel host. In this experiment each bird received only one type of louse: either 25 wing lice or 25 body lice (*n* = 6–7 birds per species for each species of louse). Coordinated transfers of ground-dove wing and body lice were not possible because they are more difficult to culture than pigeon lice.

VISUAL EXAMINATIONS

The position of the lice on each bird was recorded using a 15 region visual examination that accurately reflects the number of lice on a bird (Clayton & Drown 2001). Feather lice are relatively sedentary and restricted to the feathers; they are therefore visible and relatively easy to count on birds held in the hand. Visual censuses consisted of a careful examination of each flight feather on one wing (left) and the tail, followed by a 30-s examination of each of the following regions: underwing coverts (left side), crown, nape, gulum, neck (left side), breast, keel, side (left side), flank (left side), vent, back, anterior rump and posterior rump (Clayton 1991). For regions where only the left side was censused, the number of lice in that region was doubled for analyses because the distribution of feather lice tends to be similar on the two sides (pers. obs.).

For the analyses, I recognized six major microhabitats, each of which included up to five of the visually examined regions. As wing lice and body lice tend to occupy different regions of the host (Nelson & Murray 1971; Clayton 1991; Bush & Malenke 2008), different microhabitats were recognized for the two types of lice. For *Columbicola*, regions of the wing and tail were more finely subdivided (Fig. 2a); for Physconelloidinae abdominal regions were subdivided (Fig. 2b). Microhabitat distributions were analyzed with Repeated Measures ANOVAs in STATVIEW 5·0·1 (SAS Institute Inc. 1998), with the Repeated Measure being the six microhabitats observed per bird; birds with less than five lice were excluded from analysis.

MICROHABITAT DISTRIBUTION OF LICE ON WILD VS. CAPTIVE DOVES

In order to maximize the number of lice observed, I experimentally impaired preening in all of the captive birds. Preening is a bird's primary defence against ectoparasites. Birds with naturally or experimentally impaired preening have many more lice than birds that can preen normally (Clayton *et al.* 1999, 2005). I impaired preening by inserting a small C-shaped plastic bit between the upper and lower mandibles

of the bill. Bits spring shut slightly in the nostrils to prevent dislodging, but they do not damage the tissue and have no other apparent side effects (Clayton 1991). Bits create a 1–3 mm gap between the mandibles that impairs the forceps-like action of the bill required to efficiently remove lice from feathers (Clayton *et al.* 2005). Even so, bitted birds still go through the motions of regular preening (pers. obs.). Preliminary observations suggested that the distribution of lice on bitted birds did not differ appreciably from that on normally preening birds.

To test this assumption, I compared the microhabitat distribution of lice on bitted captive birds to that on wild birds with non-manipulated bills. I used freshly caught wild common ground-doves for this comparison. I captured 50 birds with mist nets 24–27 May 2001 at Rio Hondo and Laguna Atascosa, Texas. Birds were removed from the mist nets and the microhabitat distribution of their lice was immediately quantified in the field. The microhabitat distributions of lice on these wild birds were compared to that on bitted common ground-doves in the second transfer experiment.

Of the 50 wild caught common ground-doves, many had fewer than five lice and were thus excluded from the analyses. Twenty birds had ≥ 5 wing lice, and 7 birds had ≥ 5 body lice. The distribution of lice on these wild doves was compared to that on captive doves with bits (*n* = 6 with wing lice; *n* = 7 with body lice). Over the six microhabitats examined, there was no significant difference in the microhabitat distribution of wing lice on wild doves with normal preening vs. captive doves with bits (Repeated Measures ANOVA – interaction between bill treatment and microhabitat region: df = 5, 120; $F = 0.99$; $P = 0.43$; power = 0·98 given an effect size of $f = 0.4$ (Cohen 1988) based on the large effect observed by Tompkins & Clayton (1999) in their study of the microhabitat distribution of lice on swiftlets. Similarly, the microhabitat distribution of body lice did not differ significantly between wild doves with normal preening abilities and captive doves with bits (Repeated Measures ANOVA – interaction between bill treatment and microhabitat region df = 5, 60; $F = 0.79$; $P = 0.56$; power = 0·79; effect size $f = 0.4$). In summary, the microhabitat distribution of lice on captive bitted birds was representative of the microhabitat distribution of lice on wild preening birds.

Results

TRANSFERS OF PIGEON LICE TO SMALLER NOVEL HOSTS

Pigeon wing lice (*C. columbae*) were present on all five host species 2 months after being transferred (Table 1). However,

Table 1. Mean number of lice observed on different species of hosts in the transfer experiments (hosts listed from largest to smallest)

Louse	Host (*n*)	Mean	SE	Range
Rock pigeon wing louse *Columbicola columbae*	Rock pigeon (9)	25·0	4·9	8–58
	Band-tailed pigeon (12)	57·8	11·7	18–146
	White-tipped dove (5)	16·6	4·6	6–28
	Mourning dove (6)	22·0	5·5	8–41
	Common ground-dove (6)	10·7	3·4	3–26
Rock pigeon body louse *Campanulotes compar*	Rock pigeon (9)	49·8	8·2	19–99
	Band-tailed pigeon (12)	84·5	14·8	12–203
	White-tipped dove (5)	44·8	14·0	23–97
	Mourning dove (6)	104·2	19·4	53–153
	Common ground-dove (6)	24·3	4·7	12–41
Common ground-dove wing louse *Columbicola passerinae*	Rock pigeon (6)	0·2	0·2	0–1
	White-tipped dove (6)	0·3	0·3	0–2
	Mourning dove (6)	39·2	17·5	0–99
	Zebra dove (6)	58·2	15·9	18–123
	Common ground-dove (6)	88·3	28·0	5–180
Common ground-dove body louse *Physconelloides eurysema*	Rock pigeon (6)	0·5	0·5	0–3
	White-tipped dove (6)	0	0	0–0
	Mourning dove (6)	17·0	8·3	0–81
	Zebra dove (7)	22·3	12·0	0–84
	Common ground-dove (7)	53·9	20·4	8–162

the lice shifted their microhabitat distributions significantly on novel host species (Repeated Measures ANOVA – interaction between host species and microhabitat region df = 20, 160; $F = 4·80$; $P < 0·0001$). Indeed, this was the case on all four novel host species (Fig. 3). Rock pigeon wing lice shifted consistently from the wings to the heads of novel hosts, and the magnitude of this shift increased as host size decreased. The proportion of wing lice on the head of the host was negatively correlated with host size (linear equation $y = 165·9 − 22·8x$; df = 1, 4; $F = 33·4$, $P = 0·01$, sequential Bonferroni $P = 0·05$; $R^2 = 0·92$), while the proportion of wing lice on the remiges was positively correlated with host size (linear equation $y = −69·5 + 18·9x$; df = 1, 4; $F = 103·5$, $P = 0·002$, sequential Bonferroni $P = 0·01$; $R^2 = 0·97$). On common ground-doves, the smallest novel host, only 3% of wing lice were observed on the remiges of the wing, and 71% of wing lice were observed on the head. In contrast, on rock pigeons, the native host, 46% of wing lice were on the remiges of the wing, and only 19% were on the head. There were no significant relationships between the distribution of wing lice and host body size among the other microhabitats (linear regression, $P ≥ 0·20$ in all cases). In summary, as host size decreased, wing lice shifted significantly toward the head and away from the remiges (Fig. 3).

Pigeon body lice (*C. compar*) were also present on all five host species 2 months after being transferred (Table 1). However, unlike wing lice, body lice did not shift their microhabitat distributions significantly on novel host species (Repeated Measures ANOVA – interaction between host species and microhabitat region df = 20, 165; $F = 1·19$; $P = 0·27$; Fig. 3).
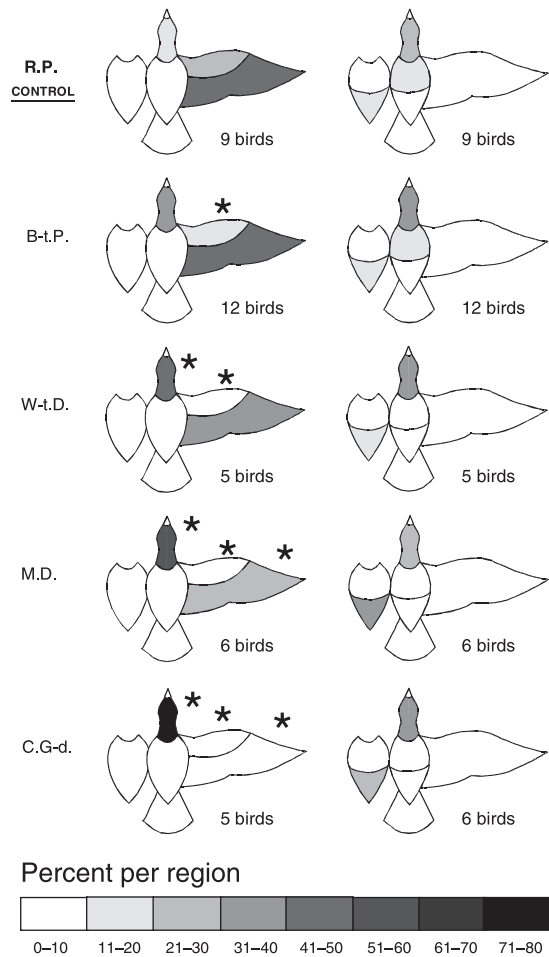
Fig. 3. Microhabitat distributions of rock pigeon lice on the native host (control) and four novel host species (host abbreviations as in Fig. 1). Shading indicates the mean percentage of lice in each microhabitat on each host species. Wing lice (*Columbicola columbae*) shifted away from the wings and onto the heads of smaller novel hosts (*$P < 0·05$, post-ANOVA Fisher PLSD tests by region). In contrast, body lice (*Campanulotes compar*) did not shift microhabitat significantly.

TRANSFERS OF GROUND-DOVE LICE TO LARGER NOVEL HOSTS

Two months after transfer, common ground-dove wing lice (*C. passerinae*) had failed to establish viable populations on the two largest novel host species, the rock pigeon and white-tipped dove (Table 1), possibly because of difficulties locating mates (Bush & Clayton 2006). Wing lice did establish on the two smaller novel hosts, the mourning dove and zebra dove, and they shifted microhabitat significantly on these hosts (Repeated Measures ANOVA – interaction between host species and microhabitat region df = 10, 65; $F = 2·04$; $P = 0·04$). Wing lice shifted significantly from the head of the smallest (native) host to the remiges of the larger novel hosts
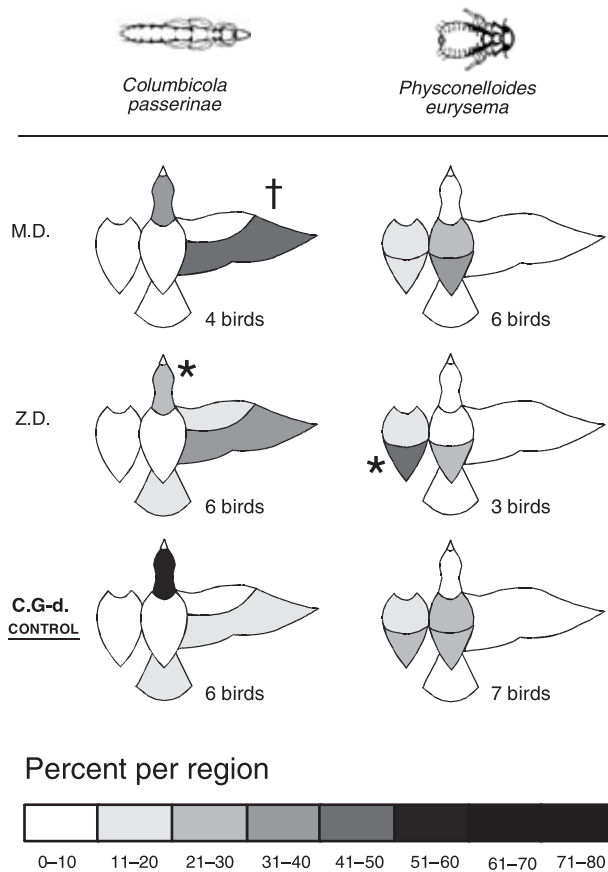
Fig. 4. Microhabitat distribution of common ground-dove lice on the native host (control) and two novel host species (host abbreviations as in Fig. 1). Shading indicates the mean percentage of lice in each microhabitat on each host species. Wing lice (*Columbicola passerinae*) shifted away from the head and onto the wings of larger novel hosts (*$P < 0.05$, †$P = 0.056$, post-ANOVA Fisher PLSD tests by region). Body lice (*Physconelloidies eurysema*) shifted microhabitat on one novel host species, but not the other.

(Fig. 4). The majority of wing lice (51%) were on the head of the native host (common ground-doves), but on zebra doves and mourning doves the percentage of lice on the head was smaller (22% and 37% respectively). In contrast, only 17% of wing lice were on the remiges of common ground-doves, while this percentage increased on zebra doves (34%) and mourning doves (49%).

As in the case of wing lice, common ground-dove body lice (*P. eurysema*) failed to establish viable populations on the two largest novel host's species (Table 1). However, they did establish on the two smaller novel hosts, the mourning dove and zebra dove. The microhabitat distribution of *P. eurysema* differed among novel host species significantly (Repeated Measures ANOVA – interaction between host species and microhabitat region df = 10, 65; $F = 2.05$; $P = 0.04$). This difference was due to the significantly large proportion of body lice on the posterior dorsal body region of zebra doves, relative to common ground-dove controls (Fig. 4). Interestingly, however, there was no significant difference in the microhabitat distribution of body lice on the even larger

bodied mourning dove relative to the common ground-dove controls (Fig. 4).

Discussion

In this study, I used a host parasite system to determine how parasites persist in novel environments. Bush & Clayton (2006) transferred lice among several species of pigeons and doves that differed in size by nearly an order of magnitude. They showed that wing and body lice from common ground-doves established on all but the two largest novel host species, and that wing and body lice from rock pigeons established on all of the novel host species to which they were transferred. Moreover, Bush & Clayton (2006) showed that wing and body lice persisted for two generations on novel host species that could preen normally, albeit in smaller populations than on bitted hosts. Invasive species often persist as small populations in a novel environment before undergoing rapid population growth and range expansion (Ghalambor *et al.* 2007). Thus, the pigeon/dove-louse system provides an ideal opportunity to study factors determining the establishment of species in novel habitats. This aim of this study was to determine if behavioural flexibility facilitates host switching in parasites, just as behavioural flexibility facilitates the ability of free-living animals to invade novel habitats (Holway & Suarez 1999; Yeh & Price 2004). To investigate whether behavioural flexibility contributes to the ability of lice to establish on novel hosts, I compared the microhabitat use of wing lice and body lice on novel host species of different sizes 2 months after experimentally transferring lice to these hosts, and to native host controls.

Wing lice (*Columbicola* spp.) consistently shifted their microhabitat distributions on different sized novel hosts, whereas body lice did not. The shift by wing lice was correlated with host size. On the smallest host species wing lice were most frequently found on the head, whereas on the largest host species wing lice were most frequently found on the flight feathers of the wings (remiges). On intermediate sized hosts wing lice were found in intermediate positions. This pattern was true for both species of wing lice, despite being from very different sized native hosts. The phrase 'wing lice' is misleading and should, perhaps, be abandoned.

Unlike wing lice, neither species of body louse exhibited consistent shifts in their microhabitat distribution. Rock pigeon body lice (*C. compar*) did not shift microhabitat significantly on any of the novel host species to which they were transferred. Common ground-dove body lice (*P. eurysema*) did experience a significant shift in microhabitat on one novel host species but not the other (Fig. 4).

The distribution of body lice makes sense in light of their feeding strategy. Body lice feed on the downy portions of the abdominal contour feathers (Nelson & Murray 1971; Clayton 1991; Bush & Malenke 2008), and they are distributed throughout the regions where they feed. Differences in the microhabitat use of body lice on novel hosts, such as the abundance of common ground-dove body lice on the lower dorsal region of the zebra doves, may simply correlate with

the relative abundance of food. The lower dorsal feathers of the zebra dove have lush downy portions (pers. obs.), which may provide lice with more food. In the future, experiments closely monitoring feather consumption might answer whether the microhabitat distribution of body lice follows an optimal foraging pattern.

The distribution of wing lice is more complex than that of body lice. Although wing lice feed on the same feathers as body lice (Bush & Malenke 2008), they also spend a considerable amount of time on the wings, tail and head, where the feathers are too coarse to eat (Nelson & Murray 1971). The distribution of wing lice in regions away from food has long been considered to be a result of competition with body lice (Clay 1949). This hypothesis was recently confirmed by Bush & Malenke (2008), who showed that body lice competitively suppress wing lice. However, interspecific competition cannot explain the observed shifts in microhabitat distribution in this study. In the first experiment, rock pigeon wing and body lice were transferred together. In this transfer interspecific competition for resources would have been most severe on the smallest novel host. Yet, on small hosts wing lice shifted away from the wings, a microhabitat that is free of body lice. In the second transfer experiment wing and body lice from common ground-doves were transferred separately. Thus, interspecific competition could not have contributed to the microhabitat shifts observed in this experiment, but wing lice shifted their microhabitat distribution on novel hosts, anyway. Moreover, wing lice from common ground-doves and rock pigeons shifted in similar patterns regardless of the presence of body lice.

In addition to being a refuge away from body lice, the microhabitats of the wings, tail and head offer a refuge from host defence. A bird's principle defence against ectoparasites is preening. Because a bird cannot preen its head, lice on the head are protected. On the flight feathers of the wings and tail, wing lice escape from preening by inserting between the furrows of adjacent feather barbs (Bush, Sohn & Clayton 2006). One disadvantage of being on the head may be increased exposure to harmful UV rays (Cywinska, Crump & Lean 2000). Behavioural avoidance of UV is known among other insects; for example, thrips exposed to UV shift to more shaded host plants (Mazza *et al*. 1999). All else being equal, wing lice may prefer the underside of the wing because it offers refuge from other lice, preening and UV radiation.

Why then do rock pigeon wing lice move to the head of smaller hosts? The ability of rock pigeon wing lice to insert between the barbs of wing feathers is compromised on the flight feathers of smaller novel hosts (Bush *et al*. 2006). The lice are simply too large (metathorax width = 304 μm; Johnson *et al*. 2005) to fit between the interbarb spaces of the smaller host species. The width of the interbarb space of common ground-dove flight feathers is only 198 μm, and that of the mourning dove is only 252 μm (Johnson *et al*. 2005; Bush *et al*. 2006). Thus, the shift to the head is a shift to feathers that offer another kind of protection from host defence because the host simply cannot preen this region.

It is also worth noting that wing lice shifted their oviposition sites on novel hosts. Typically wing lice from rock pigeons glue their eggs in tidy rows in the furrows between barbs on the covert feathers. Rock pigeon wing lice that were transferred to intermediate sized novel hosts, such as the mourning dove, continued to lay eggs on the covert feathers, but the eggs were no longer laid in rows. As the eggs were too big to fit snugly in the narrower furrows, they were attached more precariously to the tops of the furrows, which may make them more susceptible to removal by preening. When transferred to the smallest novel host, the lice abandoned the coverts and instead glued their eggs to the feathers on the head (pers. obs.). Wing lice from rock pigeons may have laid eggs on the heads of common ground-doves simply because of their inability to escape from host defence on the wings (Waage 1979; Marshall 1981). Alternatively, the lack of suitable oppositioning sites on the wings may have contributed to the shift of adult wing lice to the head.

The distribution of wing lice from common ground-doves is perplexing, at first. Despite the general assumption that lice in the genus *Columbicola* are adapted for life on the wings (Clay 1949; Clayton *et al*. 1999), relatively few common ground-dove wing lice were found on the wing of the native host. Furthermore, insertion of lice between the barbs of flight feathers was virtually non-existent. Of the more than 800 *C. passerinae* I observed on wild and captive common ground-doves, only one was inserted between adjacent feather barbs. The wings of common ground-doves may be particularly inhospitable to lice because they are known to have an unusually vigorous take-off for flight. The short, wide wings of these doves are well adapted for explosive take-offs (aspect ratio of 1 : 59; del Hoyo *et al*. 1997), and may produce aerodynamic forces that dislodge lice. Common ground-dove wing lice can avoid these forces by being on the head. When ground-dove wing lice were transferred to mourning doves, however, 54% of them were found on the flight feathers of the wing. Of these lice, 5% were inserted between feather barbs.

The establishment of lice on novel hosts cannot be explained by phylogenetic 'inertia'. In other parasite systems establishment has been shown to decrease with host phylogenetic distance; these systems include herbivorous beetles (Futuyma & McCafferty 1990; Futuyma, Keese & Funk 1995), gopher lice (Reed & Hafner 1997), mammal fleas (Krasnov *et al*. 2004), acanthocephalan worms of cockroaches (Moore & Gotelli 1996), parasitoid-flies of ants (Morehead & Feener 2000) and nematodes of *Drosophila* (Perlman & Jaenike 2003). In this study, the microhabitat shifts experienced by rock pigeon wing lice on smaller novel hosts were correlated with an increase in host phylogenetic distance (Fig. 1); however, in the second experiment, the transfers of lice from small to large hosts were independent of host phylogenetic distance (Fig. 1). In these transfers the wing lice also experienced microhabitat shifts on different sized novel hosts, suggesting that the ability of wing lice to shift microhabitats is vital to their establishment on different sized novel hosts, regardless of host relatedness.

Harrison's Rule, which is the correlation between parasite body size and host body size, does not preclude parasites from switching to different sized novel hosts. Wing lice, which adhere to Harrison's rule, were able to cope with novel environments by shifting to different microhabitats on different sized novel host species. Baldwin (1896) suggested that behavioural flexibility buys time for organisms to persist in novel environments. In turn, time buys opportunities for the accumulation of genetic changes that allow the organism to become adapted to the new environment (Price *et al.* 2003b). In this system, the Baldwin effect may allow wing lice time to adapt to new body size optimums on novel hosts. Consequently, it is possible for wing lice to exhibit strong correlations with host body size over macroevolutionary time, while still being able to switch to different sized novel hosts, as has happened periodically in the history of this parasite (Johnson & Clayton 2003).

Acknowledgements

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