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THE ROLE OF BODY SIZE IN HOST SPECIFICITY: RECIPROCAL TRANSFER EXPERIMENTS WITH FEATHER LICE

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Abstract.—Although most parasites show at least some degree of host specificity, factors governing the evolution of specificity remain poorly understood. Many different groups of host-specific parasites show a striking correlation between parasite and host body size, suggesting that size reinforces specificity. We tested this hypothesis by measuring the relative fitness of host-specific feather lice transferred to pigeons and doves that differ in size by an order of magnitude. To test the general influence of size, we transferred unrelated groups of wing and body lice, which are specialized for different regions of the host. Lice were transferred in both directions, from a large native host species, the rock pigeon (*Columba livia*), to several progressively smaller hosts, and from a small native host species, the common ground dove (*Columbina passerina*), to several larger hosts. We measured the relative fitness (population size) of lice transferred to these novel host species after two louse generations. Neither wing lice nor body lice could survive on novel host species that were smaller in size than the native host. However, when host defense (preening behavior) was blocked, both groups survived and reproduced on all novel hosts tested. Thus, host defense interacted with host size to govern the ability of lice to establish on small hosts. Neither wing lice nor body lice could survive on larger hosts, even when preening was blocked. In summary, host size influenced the fitness of both types of feather lice, but through different mechanisms, depending on the direction of the transfer. Our results indicate that host switching is most likely between hosts of similar body size. This finding has important implications for studies of host-parasite coevolution at both the micro- and macroevolutionary scales.

Key words.—Bird, coevolution, Columbiformes, parasite, specialization.

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Body size has a profound effect on the form and function of living organisms, and it is one of the most direct links between macroevolution and microevolution (Peters 1983; Calder 1984; Jablonski 1996; Brown and West 2000). Body size can influence community-level traits, such as species diversity (Maurer et al. 1992), and it can mediate interactions between species, such as those between parasites and their hosts (Combes 2001; Morand and Poulin 2002). Indeed, selective constraints imposed by host body size may play an important role in the adaptive radiation of parasites, contributing to their impressive biodiversity (Price 1980). The importance of size is illustrated in many groups of host-specific parasites by positive across-species correlations between host and parasite body size. In short, bigger hosts often have bigger parasites. Such correlations occur within parasitic worms, crustaceans, fleas, flies, lice, and ticks as well as in herbivorous aphids, thrips, beetles, flies, moths, and flower mites (Harvey and Keymer 1991; Kirk 1991; Thompson 1994; Poulin 1998; Sasal et al. 1999; Morand et al. 2000; Johnson et al. 2005).

Correlated size of parasites and their hosts, first recognized by Harrison (1915), was dubbed ''Harrison's Rule'' by Eichler (1963) and Marshall (1981). The first phylogenetically independent comparisons to demonstrate Harrison's Rule were by Harvey and Keymer (1991) for animal parasites, and by Kirk (1991) for herbivorous insects (and animal parasites). Harrison's Rule is presumably the macroevolutionary result of prolonged adaptation by parasites to selection imposed by host size. If this is true, then parasites dispersing to hosts of the ''wrong'' size should experience reduced fitness. Conversely, the fitness of parasites that violate Harrison's Rule should be relatively independent of host size, all other things being equal.

In this paper we compare the influence of host size on the relative fitness of two groups of feather lice that coexist on Columbiform birds: wing lice, which follow Harrison's Rule, and body lice, which do not (Johnson et al. 2005). We measured the fitness (population size after two generations) of wing and body lice transferred to novel hosts varying in size by an order of magnitude, relative to the fitness of control lice transferred to new individuals of the native host. We transferred both kinds of lice in both directions (Fig. 1); that is, we transferred host-specific wing and body lice from a large-bodied host to progressively smaller hosts, and we transferred host-specific wing and body lice from a small-bodied host to progressively larger hosts. To test the impact of host defense on lice transferred to different hosts, we blocked preening in half the birds of each species. We also measured two components of louse performance—feeding ability and the ability to escape from simulated preening—using detached feathers from novel host species.

Background

Columbiform wing and body lice are ecological replicates (Johnson and Clayton 2003) that provide a powerful comparative context for testing the adaptive basis of Harrison's Rule. Both groups are permanent ectoparasites that complete their entire life cycle on the body of the host (Marshall 1981). The three- to four-week life cycle begins with the egg, which is glued to the feathers, then progresses through three nymphal instars, to the adult stage. Both groups feed primarily on feathers, which are metabolized with the aid of endosymbiotic bacteria. 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

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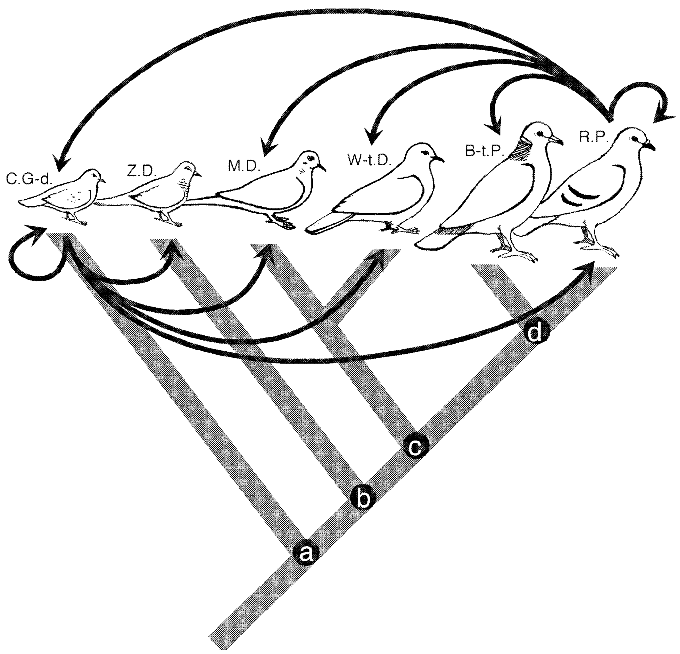

FIG. 1. Design of parasite transfer experiments. Host species (drawn to scale) varied in size by nearly an order of magnitude (mean body mass of actual birds used in experiments): C.G-d., common ground dove (*Columbina passerina*, 45 g); Z.D., zebra dove (*Geopelia striata*, 50 g); M.D., mourning dove (*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) wing and body lice from rock pigeons were transferred to progressively smaller novel host species and to rock pigeon controls. In the second experiment (bottom arrows) wing and body 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 gray (Clayton et al. 2003a; K. P. Johnson, unpubl. 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.

have both wing and body lice (Price et al. 2003). Both groups of lice exhibit significant phylogenetic congruence with their hosts worldwide, revealing a long history of parasite-host association (Johnson and Clayton 2003). The terms ''pigeon'' and ''dove'' are informal references to relative body size (large and small) with no formal taxonomic meaning; hereafter, we refer to all hosts as doves for simplicity.

Johnson et al. (2005) reported correlated evolution of parasite and host body size across a diverse group (45 genera) of host-specific bird lice (Insecta: Phthiraptera). Among bird lice, the adaptive basis of Harrison's Rule has been documented in the case of dove wing lice, which escape from host defense (preening) by hiding in the furrows between barbs of the wing and tail feathers (Clayton 1991). On the native host species, preening exerts selection on wing louse size, improving the match to furrow size (Clayton et al. 1999). Across host species, preening exerts selection against wing lice experimentally transferred to small novel hosts because the lice cannot hide as well in the smaller furrows (Clayton et al. 2003a). Selection for small size on such hosts may be balanced by selection for large size because of the correlation between size and fecundity in insects (Sibly and Calow 1986). These opposing selective forces should reinforce the fit to Harrison's Rule shown by dove wing lice (Johnson et al. 2005).

Curiously, dove body lice are unusual among bird lice in violating Harrison's Rule (Johnson et al. 2005); indeed, similar-sized body lice are found across the full range of dove body sizes. Body lice escape from host preening by burrowing into the downy regions of abdominal feathers (Clayton 1991). This form of escape may be independent of feather size, which could explain why body lice do not follow Harrison's Rule. We predict that, unlike wing lice, the fitness of body lice on novel hosts should be independent of body size. A major goal of the current project was to test this prediction.

MATERIALS AND METHODS

We tested the ability of lice to establish viable populations on host species of different sizes by transferring them from the native host to novel hosts and to (control) individuals of the native host (Fig. 1). We measured the impact of host defense on establishment by blocking preening in 50% of the birds in each experimental treatment. We 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. They create a 1–3-mm gap between the mandibles that impairs the forcepslike action of the bill required for efficient preening (Clayton et al. 2005). Bits do not interfere with feeding and they have no other apparent side effects (Clayton et al. 1999).

All birds were wild individuals trapped with nets or walk-in traps at the following localities (see Fig. 1 legend for scientific names): common ground doves and white-tipped doves, Hidalgo County and Cameron County, Texas; zebra doves, Honolulu County, Hawaii; mourning doves, Pima County, Arizona; band-tailed pigeons, Benton County, Oregon; rock pigeons, Salt Lake County, Utah. Birds were housed individually in 30 × 30 × 56 cm wire mesh cages in our animal facility. Cages were separated with Plexiglas partitions to prevent transmission of lice via contact between birds in adjacent cages. 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. This method kills lice and eggs while avoiding residues that might result from the use of chemical fumigants (Moyer et al. 2002a). Relative humidity in the animal rooms was elevated to an optimum for lice (60–70%) during the transfer experiments (Nelson and Murray 1971; Moyer et al. 2002a).

Transfer experiments lasted two months, which is more than two louse generations (Martin 1934; Price et al. 2003). At the end of each experiment the birds were euthanized and their louse populations determined by body washing, which accounts for 99% of the lice on a bird (Clayton and Drown 2001). The number of lice on each bird was determined using

$y = [1.10(x^{1/2})]^2$ for wing lice, and $y = [1.05(x^{1/2})]^2$ for body lice, where x was the number of lice recovered by washing. These equations were derived from reanalysis of washing data in Clayton and Drown (2001), but with the regressions forced through the origin ($R^2 = 0.99$; $P < 0.0001$ for both wing and body lice; Bush et al. 2006a). Louse population data were normalized using ln(louse population + 1) transformations prior to analysis. Because the population dataset included both adult and immature lice, it incorporated both the survival and reproductive components of parasite fitness.

Transfers of Lice to Smaller Novel Hosts

In the first experiment, we transferred wing lice (*Columbicola columbae*) and body lice (*Campanulotes compar*) from the largest host species, the rock pigeon, to four progressively smaller novel host species and to native host controls (Fig. 1, top arrows; $n = 12$ birds per species, except white-tipped dove, $n = 10$). 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 serves as a positive control for comparing the fitness 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 a typical population size on captive rock pigeons with normal preening ability (Clayton et al. 2003a, 2005). Transferred lice were obtained from culture stocks bred on wild-caught rock pigeons. The lice were removed from culture birds by anesthetizing them with CO_2 in a fumigation chamber (Clayton and Drown 2001; Moyer et al. 2002b). 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 its cage.

Transfers of Lice to Larger Novel Hosts

In the second experiment, we transferred wing lice (*Columbicola passerinae*) and body lice (*Physconelloides eurysema*) from the smallest host species, the common ground dove, to four progressively larger hosts and to native host controls (Fig. 1, bottom arrows; $n = 24$ birds per species, except zebra dove and common ground dove, $n = 26$). The smallest of the four novel hosts, the zebra dove, is similar in size to the common ground dove (<10% difference in body mass). It served as a positive control for comparing the fitness of common ground–dove lice transferred to a similar-sized novel host. This experiment required larger host samples because each bird received only one type of louse: 25 wing lice or 25 body lice. Coordinated transfers of ground-dove wing and body lice are difficult because they are harder to culture in large numbers than pigeon lice.

Performance of Lice on Novel Hosts

Escape from simulated preening.—We measured the ability of *C. columbae* wing lice and *C. compar* body lice to escape from simulated preening on detached feathers. Wing lice escape from preening by hiding in furrows between the adjacent barbs of the large flight feathers on the wings and tail (Clayton 1991). Wing lice were placed one at a time in the center of a fifth primary wing feather from one of four novel host species, or from a rock pigeon control ($n = 30$ lice per host species, except band-tailed pigeon $n = 25$ lice). We used feathers from 15 different individuals of each host species. We simulated preening by stroking the feather with a brush and we categorically scored whether each louse inserted fully (1.0), about halfway (0.5), or not at all (0.0).

Body lice escape from preening by burrowing into the downy matrix of the host's abdominal contour feathers, like the feathers on the rump (Clayton 1991). To measure the escape performance of body lice, we placed three individuals in the center of a rump feather from one of four novel host species, or a rock pigeon control ($n = 3$ lice per trial, 15 trials per host species). We again used feathers from 15 different individuals of each host species. Removal trials were conducted randomly with regard to host species. After the lice burrowed into the downy matrix of the feather, we measured the time required for an assistant, blind to treatment, to remove all three lice with forceps as rapidly as possible.

Feeding ability.—Clayton et al. (2003a) tested the ability of *C. columbae* to feed on feathers from novel hosts in vitro by weighing feathers before and after feeding trials to calculate the amount of feather mass removed by lice. We tested the ability of the three other species of lice used in this study to feed on feathers in vitro. All of these species feed on abdominal contour feathers (Nelson and Murray 1971). For each of the three species of lice we plucked abdominal contour feathers from each novel host species, and from the native host. The feathers were placed in 50-ml glass tubes, with 3 feathers per tube and 15 tubes per host species (from 15 individual birds). The tubes were placed in a stainless-steel lined Percival incubator [Percival Scientific, Perry, IA) kept at 33°C and 75% relative humidity on a 12-hour light/dark photoperiod (Clayton et al. 2003a). After 24 h the feathers were removed from each tube, weighed to the nearest 0.1 mg three times on an analytical balance, and returned to the tubes. Starting feather mass was taken as the mean value of the three weights.

Soon after the feathers were weighed, 10 randomly chosen lice of a given species were added to each tube, which was then returned to the incubator for the duration of the experiment (one month). Each week the number of live lice in each tube was tallied. At the end of the experiment lice were removed from the tubes, and the feathers in all tubes were removed and weighed again three times. Change in feather mass was calculated by comparing the mean mass of feathers at the start of the experiment to mean mass at the end of the experiment (Clayton et al. 1999, 2003a).

In addition to the tubes containing feathers and lice described above, we prepared tubes containing feathers but no lice in order to quantify any changes in feather mass due to factors other than lice. We included $n = 2$ tubes per host species during the *C. compar* feeding trials, and $n \geq 3$ tubes per host species during the *C. passerinae* and *P. eurysema* trials.

We also prepared tubes containing lice, but no feathers ($n = 10$ lice per tube, 5 tubes per louse species). All of the lice in these tubes died within a few days, confirming the critical need for a steady food supply.

TABLE 1. Two-way ANOVAs testing the effects of host species and host defense (preening treatment) on the fitness of lice in the transfer experiments.

	df	*F*-ratio	*P*-value
Rock pigeon lice			
Columbicola columbae			
Host species	4, 48	9.28	≤0.0001
Preening treatment	1, 48	147.46	≤0.0001
Interaction	4, 48	3.15	0.022
Campanulotes compar			
Host species	4, 48	7.23	≤0.0001
Preening treatment	1, 48	325.68	≤0.0001
Interaction	4, 48	3.86	0.009
Common ground–dove lice			
Columbicola passerinae			
Host species	4, 50	24.51	≤0.0001
Preening treatment	1, 50	19.30	≤0.0001
Interaction	4, 50	1.59	0.191
Physconelloides eurysema			
Host species	4, 54	15.89	≤0.0001
Preening treatment	1, 54	61.60	≤0.0001
Interaction	4, 54	5.32	0.001

RESULTS

Transfers of Lice to Smaller Novel Hosts

We compared the relative fitness (population size) of rock pigeon lice transferred to novel hosts of different sizes with and without normal preening ability. Host species and preening treatment each had a significant effect on the fitness of both wing and body lice ($P \leq 0.0001$, Table 1). The interaction between host species and preening was also significant for wing and body lice ($P \leq 0.022$), showing that the impact of preening depended on the host species to which lice were transferred. Preening affected lice to varying degrees on all five species of hosts (filled squares, Fig. 2a,b). However, its effect was greatest on the three smallest species; populations of both wing and body lice were reduced to near zero on common ground doves, mourning doves, and white-tipped doves. In contrast, populations of wing and body lice on band-tailed pigeons were similar in size to those on rock pigeons, the native host species.

When preening was impaired, wing and body lice increased in number on all five species of hosts (open squares, Fig. 2a,b), showing that they could both survive and reproduce in the absence of host defense. The increase was smallest on common ground doves, which had means of 41 wing lice (range =17–70) and 97 body lice (range =58–195). Louse populations increased more on the other three novel host species, and did not differ significantly from those on the native rock pigeon host, which had means of 182 wing lice (range = 75–363) and 279 body lice (range = 132–645). The ability of wing and body lice to survive and reproduce on all four novel host species confirms that the feathers of these species provide adequate food and habitat for lice, at least in the absence of host defense.

Transfers of Lice to Larger Novel Hosts

We compared the relative fitness (population size) of common ground–dove lice transferred to novel hosts of different sizes with and without normal preening ability. As in the previous experiment, host species and preening each had a significant overall effect on the fitness of both wing and body lice ($P \leq 0.0001$, Table 1). The interaction between host species and preening was significant for the body louse *P. eurysema* ($P = 0.001$, Table 1), but not for the wing louse *C. passerinae* ($P = 0.191$). Any effect of the interaction between host size and preening on wing lice was masked by the fact that wing louse populations were near zero on the two largest hosts (white-tipped doves and rock pigeons; Fig. 2c), even in the absence of host defense. This was also true for body lice on white-tipped doves (Fig. 2d).

Performance of Lice on Novel Hosts

Escape from simulated preening.—In response to simulated preening, most wing lice (62%) showed at least partial insertion between wing feather barbs. However, insertion varied considerably among the five host species: 92% of lice were fully or partially inserted on native rock pigeon feathers, compared with 0% of lice on feathers from common ground doves. Insertion was highly correlated with host body size (Fig. 3a; $r_s = 0.645$, $P < 0.0001$, $n = 145$ lice).

Time required to remove body lice from downy feathers by simulated preening was also correlated with host size (Fig. 3b; $r = 0.35$, $P = 0.0002$, $n = 18$ trials per host species, with three lice per trial). More time was required to remove lice from the feathers of larger host species.

Feeding ability.—There was no significant difference in the survival of rock pigeon body lice (*C. compar*) in vials of feathers from the five host species ($P = 0.197$; Table 2), nor was there a significant difference in the amount of feather material they consumed ($P = 0.197$; Table 3; Fig. 4b). The overall mean decrease in feather mass in tubes with lice was 1.18 ± 0.10 mg, compared to 0.08 ± 0.03 mg in tubes without lice ($F = 17.74$, df = 1, $P < 0.0001$). Hence, decreased feather mass was caused by lice feeding on the feathers; furthermore, frass (louse feces) accumulated on the bottom of all louse-infested tubes. There was a significant decrease in louse survival over time ($P \leq 0.0001$, Table 2). This is not surprising given that one month is a substantial proportion of the life span of a louse (Martin 1934).

The results for common ground–dove lice were similar. There was no significant difference in the survival of wing lice (*C. passerinae*; $P = 0.714$; Table 2), despite the fact that, on rock pigeon feathers, they consumed significantly more than on native common ground–dove host feathers (Table 3; Fig. 4c). Tubes with lice showed an overall mean decrease in feather mass of 1.42 ± 0.09 mg, compared to 0.12 ± 0.03 mg in tubes without lice ($F = 49.52$, df = 1, $P < 0.0001$). These decreases were accompanied by an accumulation of frass.

Common ground–dove body lice (*Physconelloides eurysema*) showed no significant differences in survival ($P = 0.623$; Table 2) or feather consumption ($P = 0.531$; Table 3, Fig. 4d). Tubes with ground-dove body lice had an overall mean decrease in feather mass of 2.02 ± 0.13 mg, compared to 0.28 ± 0.08 mg in louse-free tubes ($F = 39.59$, df = 1, $P < 0.0001$). Again, the decreases were accompanied by frass.

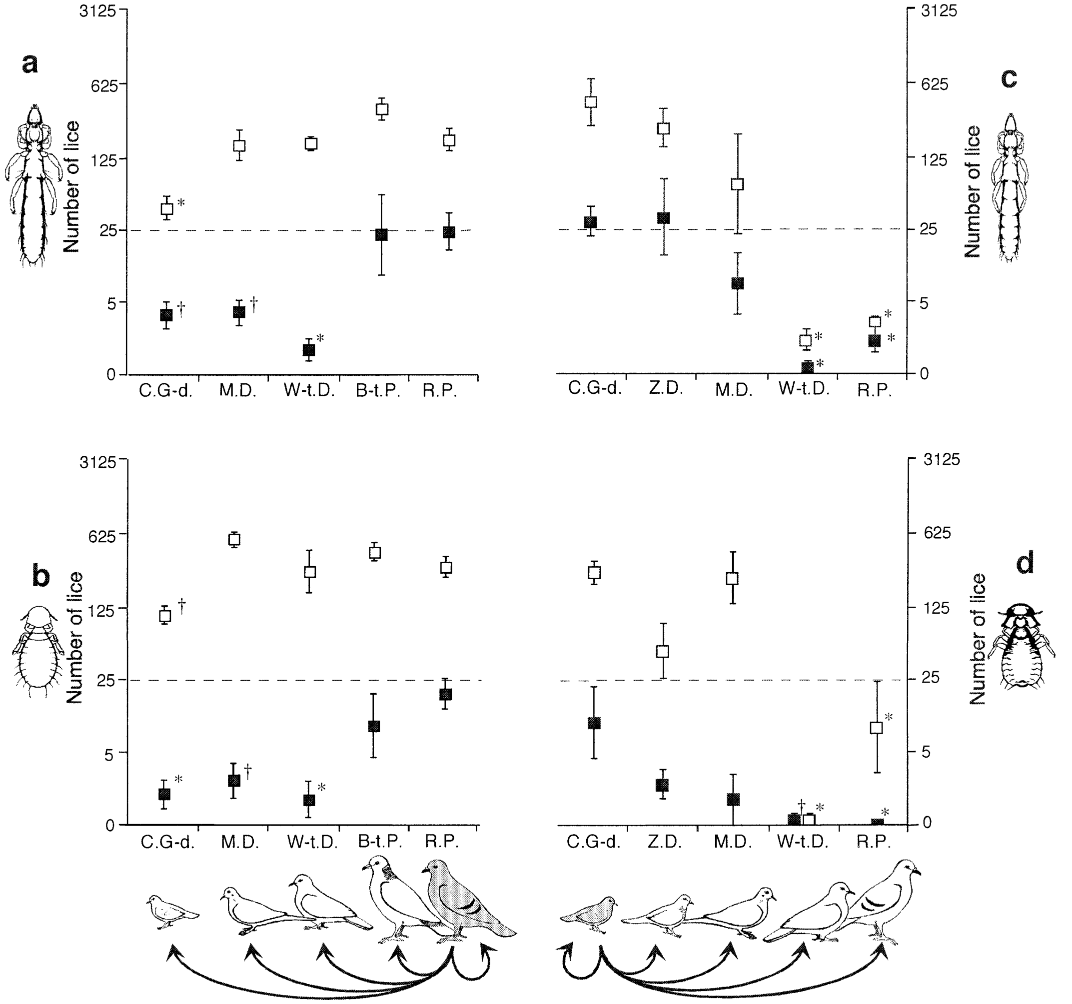

FIG. 2. Population sizes (mean ± SE) of lice transferred to novel host species relative to the native host (gray bird): (a) *Columbicola columbae*; (b) *Campanulotes compar;* (c) *Columbicola passerinae*; (d) *Physconelloides eurysema*. Host species (drawn to scale) abbreviated as in Figure 1. Note y-axes are ln scale. Open squares are lice on bitted birds that could not preen; filled squares are lice on birds with normal preening ability (n = 6 birds per square, except W-t.D. in panels (a–b), n = 5 birds per square; C.G-d. and Z.D. in panel (d), n = 7 birds per square). Dotted lines show the number of lice transferred to each individual bird at the start of the experiments, each of which was two months in duration. *$P \leq 0.01$, †$P \leq 0.05$ for Dunnett's post-ANOVA comparisons of the number of lice on each novel host species, relative to the number on native (control) hosts (within preening treatments). Data in panel (a), originally published in Clayton et al. (2003a), are repeated here for direct comparison with (b–d).

DISCUSSION

We conducted the first experimental tests of the influence of host size on the fitness of parasites that violate Harrison's Rule (body lice). For direct comparison, we also tested the influence of host size on parasites from the same hosts that follow Harrison's Rule (wing lice). Our results show that, contrary to expectation, the fitness of both groups is tied to host size. Host size influences the fitness of both types of parasites, but through different mechanisms depending on the direction of the transfer.

Transfers of Lice to Smaller Novel Hosts

Neither wing nor body lice could maintain viable populations when transferred to smaller novel hosts that could preen (Fig. 2a,b). Under these normal preening conditions, lice were only able to establish on novel hosts that were similar in size to the native host. Thus, our results indicate that the interaction of host defense and body size reinforces the specificity of both wing and body lice. Note that this decrease in parasite establishment was also correlated with an increase in host phylogenetic distance (Fig. 1). Parasite establishment has been shown to decrease with host phylogenetic distance in a variety of systems, including herbivorous beetles (Futuyma and McCafferty 1990; Futuyma et al. 1995), gopher lice (Reed and Hafner 1997), mammal fleas (Krasnov et al. 2004), acanthocephalan worms of cockroaches (Moore and Gotelli 1996), parasitoid flies of ants (Morehead and Feener 2000), and nematodes of *Drosophila* (Perlman and Jaenike 2003). In such cases, host phylogenetic distance merely serves as a surrogate measure of host similarity (Poulin 2005). The relationship of parasite establishment to host phylogeny is interesting, but host phylogeny, per se, does not reveal which host phenotypic characteristics

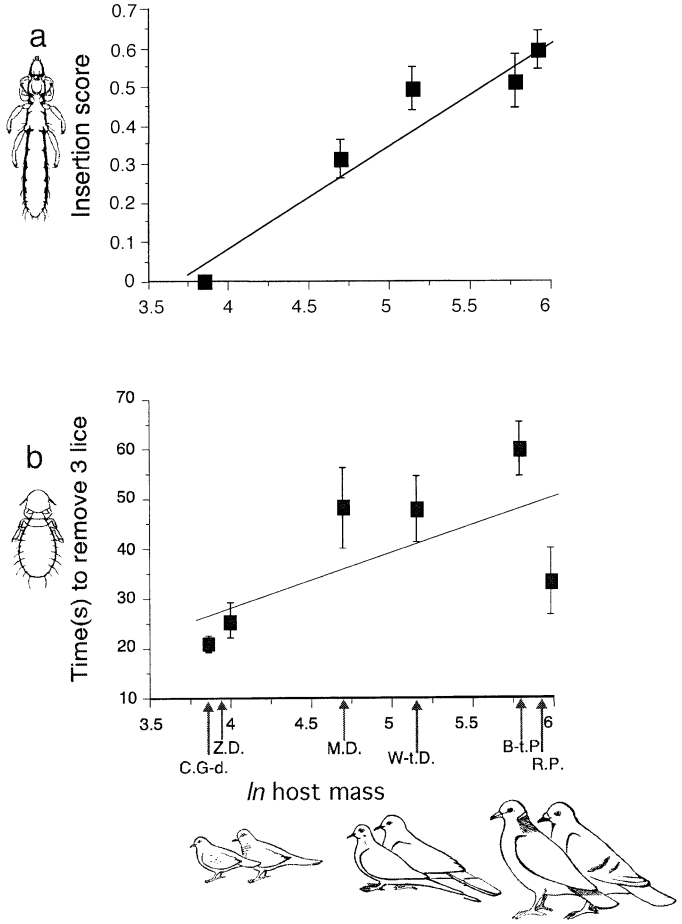

FIG. 3. Relative efficiency of escape from host defense on different host species (drawn to scale; abbreviations as in Fig. 1). (a) Mean (± SE) insertion by *Columbicola columbae* was highly correlated with host body mass; (b) mean (± SE) time to remove three *Campanulotes compar* during bouts of simulated preening was also correlated with host body mass.

govern parasite establishment. One way to expose proximal factors governing parasite establishment is to experimentally manipulate particular host characteristics. In our transfer experiments we independently manipulated host defense, decoupling the effect of preening from host phylogeny.

When preening was blocked, both wing and body lice were able to establish on all of the novel hosts, regardless of host size. Although preening reduced louse populations on all hosts, it most severely impacted lice on the smallest hosts. Thus, the effect of preening depended on the size of the host species to which lice were transferred. By the end of the two-month experiment, population sizes of wing and body lice on all but the smallest host (common ground dove) did not differ significantly from those on the native rock pigeon (Fig. 2a,b). The fact that the population sizes of lice on common ground doves were significantly less than those on the native host is not surprising, given that common ground doves are nearly an order of magnitude smaller than the native host. Food resources on common ground doves may have been limiting.

Rock pigeon lice transferred to band-tailed pigeons established as well as those on the native host, even in the face of normal preening (Fig. 2a,b). These results suggest that the band-tailed pigeon could be a suitable host for rock pigeon lice under natural conditions. Why, then, do these two species not share lice, particularly given that band-tailed pigeons and rock pigeons are broadly sympatric (del Hoyo et al. 1997)? We believe the answer is related to the fact that, although these birds are found in the same geographic regions, band-tailed pigeons live primarily in montane, forested habitat that is not typically occupied by rock pigeons (Johnston and Janiga 1995). Hence, the absence of rock pigeon lice from band-tailed pigeons may reflect a lack of ecological opportunity for lice to disperse between these two species. Furthermore, rock pigeons are a European species introduced to North America in the 1600s (Johnston and Janiga 1995), which is quite recent in macroevolutionary time. Because band-tailed pigeons are becoming increasingly common in urban areas (Keppie and Braun 2000; National Geographic 2002), we predict that they may share lice with rock pigeons at some point in the future.

In summary, preening appears to be the only real barrier to the establishment of lice on smaller novel hosts. Individual hosts vary naturally in their preening abilities (Clayton 1991). A small percentage of birds have naturally occurring minor bill deformities that impair preening (Pomeroy 1962; Clayton et al. 1999). These birds may provide a stepping-stone opportunity for parasites like lice to eventually establish on smaller novel hosts, assuming the lice are able to adapt quick-

TABLE 2. Repeated-measures ANOVAs testing the effect of host species over time on survival in the feeding experiments.

	df	*F*-ratio	*P*-value
Rock pigeon lice			
Campanulotes compar			
Host species[1]	4, 280	1.55	0.197
Time[1]	4, 280	542.10	≤0.0001
Interaction[2]	16, 280	0.78	0.707
Common ground–dove lice			
Columbicola passerinae			
Host species[1]	4, 280	0.53	0.714
Time[1]	4, 280	742.70	≤0.0001
Interaction[2]	16, 280	0.75	0.742
Physconelloides eurysema			
Host species[1]	4, 280	0.66	0.623
Time[1]	4, 280	927.12	≤0.0001
Interaction[2]	16, 280	1.48	0.107

[1] Power = 0.991; effect size, $f^2 = 0.35$ (Bush et al. 2006a).
[2] Power = 0.914; effect size, $f^2 = 0.35$.

TABLE 3. One-way ANOVAs testing the effect of host species on the amount of feather material consumed in the feeding experiments.

	df	*F*-ratio	*P*-value
Rock pigeon lice			
Campanulotes compar[1]	4, 70	1.55	0.197
Common ground–dove lice			
Columbicola passerinae[1]	4, 70	4.83	0.002
Physconelloides eurysema[1]	4, 70	0.80	0.531

[1] Power = 0.771; effect size, $f = 0.40$ (Bush et al. 2006a).

ly to the new host. The fact that louse generation time is an order of magnitude shorter than host generation time may increase the likelihood of this scenario.

Transfers of Lice to Larger Novel Hosts

Populations of both wing and body lice decreased on the two largest host species (white-tipped doves and rock pigeons), even when preening was blocked (Fig. 2c,d). Also, transfers of lice from small to large hosts were independent of host phylogenetic distance (Fig. 1). Hence, in contrast to the transfers of lice to *smaller* hosts, some factor(s) other than, or in addition to, host defense influenced the establishment of lice on the largest hosts. Common ground–dove lice on intermediate-sized mourning doves fared better, especially when preening was blocked. On birds that could preen, however, both wing and body louse populations tended to decline with increasing host size (Fig. 3c,d). This trend presumably explains why common ground–dove lice are not found on mourning doves in nature, despite the fact that the two host species are sympatric and syntopic (del Hoyo et al. 1997). These lice are not found on the zebra dove because it is an Old World species that has been introduced to Hawaii, but not North America.

Performance of Lice on Novel Hosts

Escape from simulated preening.—Preening prevented the establishment of rock pigeon lice transferred to novel hosts that were smaller in size than the native host (Fig. 2a,b). The reason is straightforward in the case of wing lice, which escape from preening by hiding in the furrows between adjacent barbs of the large flight feathers (Clayton 1991). Mean furrow size is highly correlated with overall body size among Columbiformes (Johnson et al. 2005), making it difficult for wing lice to escape by insertion on small hosts (Bush et al. 2006b). Indeed, data collected from live birds at the end of our transfer experiment showed significantly fewer lice inserted between feather barbs on the three smallest hosts, compared to the two largest hosts (visual examination method of Clayton and Drown (2001); *G*-test, $G_{adj} = 5.94$, df = 1, $P = 0.015$). These observations were corroborated by direct measures of insertion ability on detached feathers from the host species in our transfer experiment (Fig. 3a). These results indicate that wing lice on large novel hosts are fully capable of inserting between feather barbs to escape from preening. Not surprisingly, rock pigeon wing lice can fully insert on feathers from hosts an order of magnitude larger than their native host (Bush et al. 2006b).

Similarly, the escape data for body lice show that bigger feathers are better; it took significantly longer to remove body lice on feathers from larger hosts (Fig. 3b). The thick downy matrix of feathers from large hosts appears to offer greater protection than that from small hosts (S. E. Bush, pers. obs.).

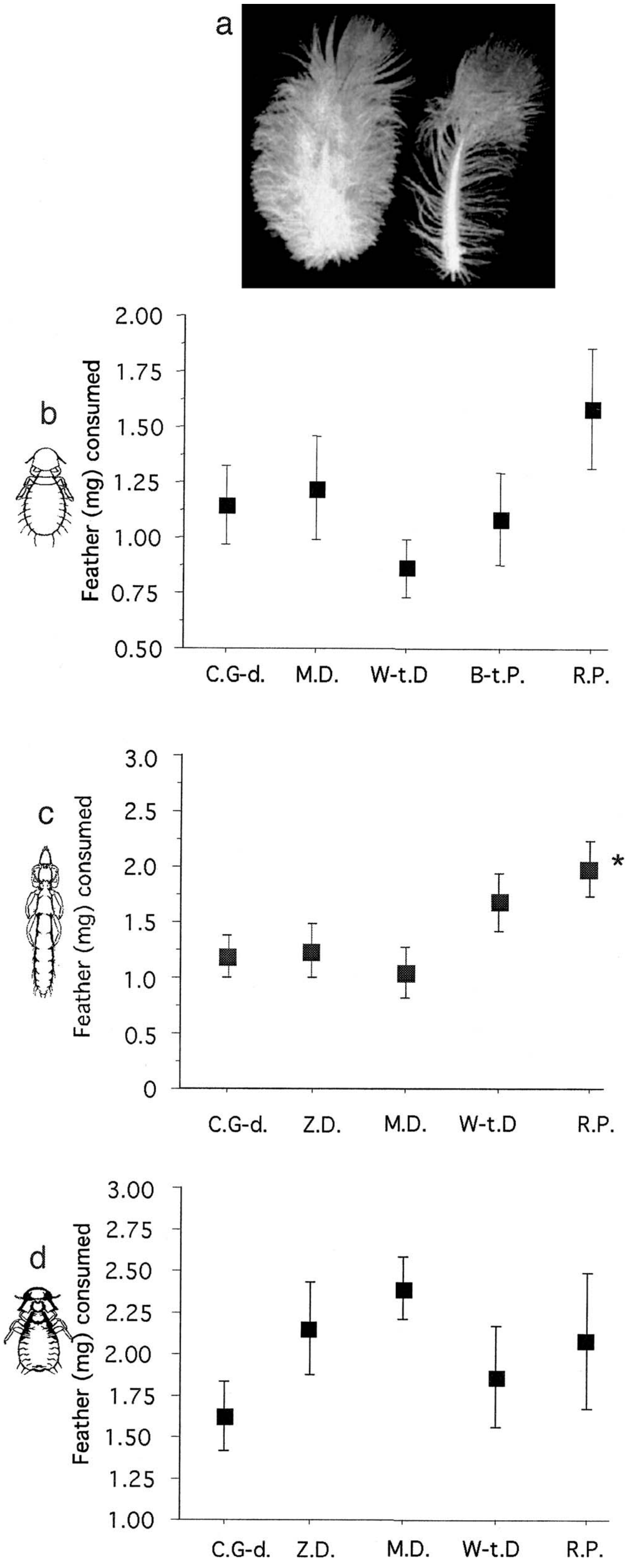

FIG. 4. (a) Abdominal contour feathers of a rock pigeon: undamaged (left) and thoroughly consumed by lice (right): (b–d) Mean (± SE) feather mass consumed by (b) rock pigeon body lice (*Campanulotes compar*), (c) common ground–dove wing lice (*Columbicola passerinae*), and (d) common ground–dove body lice (*Physconelloides eurysema*). $^{*}P < 0.01$ for Dunnett's post-ANOVA comparisons of feather consumption by lice on each novel host species, relative to that on its native (control) host. Host abbreviations as in Figure 1.

←

Thus, as in the case of wing lice, escaping from preening appears to be more difficult on smaller novel hosts.

In contrast, the poor establishment of wing and body lice on large novel hosts is not explained by an inability of these lice to escape from host defense. Our results indicate that one or more other factors are involved.

Feeding ability.—Both wing and body lice use their chewing mandibles to sever barbules of the downy regions of abdominal feathers (Fig. 4a). Conventional wisdom suggests that louse mandible size, which is correlated with overall louse body size, limits the range of feather sizes on which lice can feed (Clay 1949; Kirk 1991). However, the results of our feeding experiments provide no support for this hypothesis. There were no significant differences in feather consumption nor survival of lice cultured on feathers from small novel hosts (Fig. 4b, Table 2). Similarly, lice removed as much, if not more, feather mass from feathers of large novel hosts, relative to feathers from the native host (Fig. 4c,d). Furthermore, survival of lice on feathers from these hosts did not differ significantly from the native host (Table 2).

Why Can't Lice Establish on Large Novel Hosts?

Our results indicate that the inability of lice to establish on large novel hosts, even when preening is blocked, is not explained by obvious problems escaping from host defense (Fig. 3) nor feeding on novel host feathers (Fig. 4). Experiments with wing lice (Bush et al. 2006b) also show that lice are not simply falling off larger hosts, since they are fully capable of remaining attached to a wide variety of feather sizes. It is also unlikely that poor establishment on large hosts is an experimental artifact related to the fact that ground dove wing and body lice were transferred separately, whereas rock pigeon wing and body lice in the first experiment were transferred simultaneously to smaller hosts (see Materials and Methods). If anything, transferring lice separately to large hosts would have increased the probability of establishment because it eliminated any potential for competition between wing and body lice, which is known to occur (Clayton et al. 2003b).

Poor establishment on large novel hosts in our experiment might conceivably have been a consequence of poor parasite reproductive success (Adamson and Caira 1994). Finding mates may be easiest on small hosts where chemical cues used to locate conspecifics are presumably more concentrated. Lice are known to be attracted to conspecific feces (J. M. Clark, pers. comm.), which will be more concentrated on the smaller surface area of a smaller bodied host. In contrast, chemical cues will be diluted on larger hosts, where parasites must also travel greater distances to locate mates. Tests of these hypotheses require careful experiments that tease apart the survival and reproductive components of fitness for parasites on hosts of different body sizes.

Conclusions

Our results show that host size is a fundamental determinant of host specificity, at least in this host-parasite system. Host size matters when parasites are transferred to small novel hosts and when parasites are transferred to large novel hosts. Host size matters for parasites that follow Harrison's Rule, as well as for parasites that do not follow Harrison's Rule. The influence of host size on the fitness of feather lice, and probably other kinds of parasites, has both micro- and macroevolutionary consequences.

For example, on a microevolutionary scale, we predict that host species most at risk of acquiring parasites from invasive host species will be those that are similar in size to the invasive hosts. For example, the Eurasian collared dove (*Streptopelia decacto*), an invasive species spreading across North America (Romagosa 2002), has host-specific feather lice that are not normally found on North American doves (Price et al. 2003). It is likely that collared-dove lice will eventually disperse to native North American doves via direct contact at bird feeders, shared dust baths, phoretic hitchhiking on other more mobile parasites, or by other means (Clayton et al. 2004). However, we predict that these lice will become successfully established only on mourning doves (*Zenaida macroura*) and white-winged doves (*Z. asiatica*), which are similar in size to the Eurasian collared dove. Predicting the pattern of a successful parasite invasion has important conservation implications (Dobson and May 1986; Thorne and Williams 1988; Laurance et al. 1996). We suggest that host body size can, in cases such as feather lice, be used as a predictor of susceptibility to novel ectoparasites and may thus aid in targeting conservation efforts.

On a macroevolutionary scale, we predict that host switching is a more likely explanation for incongruent regions of host and parasite phylogenies when those regions contain host species that are similar in body size. Lice have been used more than any other group in the development of analytical methods for assessing patterns of cophylogenetic congruence and rates of cospeciation (Page 2003). Congruence between host and parasite phylogenies is affected by more than just the process of cospeciation, which increases congruence, and host switching, which erodes it. Congruence is also eroded by local extinction and other sorting events (Clayton et al. 2004). We suggest that comparisons of host body size can, in cases such as feather lice, be used to assess the relative importance of host switching in reconstructions of coevolutionary history.

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