

Reciprocal Natural Selection on Host-Parasite Phenotypes

Dale H. Clayton,^{1,*} Patricia L. M. Lee,^{1,†} Daniel M. Tompkins,^{1,‡} and Edmund D. Brodie III^{2,§}

1. Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom;

2. Center for Ecology, Evolution and Behavior, T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506-0225

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ABSTRACT: Coevolution is evolution in one species in response to selection imposed by a second species, followed by evolution in the second species in response to reciprocal selection imposed by the first species. Although reciprocal selection is a prerequisite of coevolution, it has seldom been documented in natural populations. We examined the feasibility of reciprocal selection in a simple host-parasite system consisting of feral pigeons (*Columba livia*) and their Ischnoceran feather lice (Phthiraptera: Insecta). We tested for a selective effect of parasites on hosts with experimentally altered defenses and for a selective effect of host defense on a component of parasite escape. Previous work indicates that pigeons control lice through efficient preening, while lice escape from preening using complex avoidance behavior. Our results show that feral pigeons with impaired preening, owing to slight bill deformities, have higher louse loads than pigeons with normal bills. We use a controlled experiment to show that high louse loads reduce the survival of pigeons, suggesting that lice select for efficient preening and against bill deformities. In a reciprocal experiment, we demonstrate that preening with a normal bill selects for small body size in lice, which may facilitate their escape from preening. The results of this study verify a crucial element of coevolutionary theory by identifying likely targets of reciprocal phenotypic selection between host and parasite.

Keywords: coevolution, birds, ectoparasites, lice, virulence, fitness.

* To whom correspondence should be addressed; present address: Department of Biology, University of Utah, Salt Lake City, Utah 84112; e-mail: clayton@biology.utah.edu.

† Present address: School of Biological Sciences, University of Wales, Swansea SA2 8PP, United Kingdom; e-mail: p.l.m.lee@swansea.ac.uk.

‡ Present address: Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, United Kingdom; e-mail: d.m.tompkins@stir.ac.uk.

§ Present address: Department of Biology, Indiana University, Bloomington, Indiana 47405; e-mail: bbrodie@bio.indiana.edu.

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Coevolution is generally regarded to be one of the most important processes shaping patterns of adaptation between species (Thompson 1994). It is thought to occur in response to reciprocal selection on the phenotypic traits of interacting species. Although the reciprocity of coevolution is what distinguishes it from other modes of evolution, reciprocal selection has seldom actually been measured in natural populations. There are a handful of notable exceptions. Davies and Brooke (1989a, 1989b) demonstrated reciprocal selection between brood-parasitic cuckoos, which lay mimetic eggs, and their avian hosts, which attempt to eject those eggs. The dynamics of gene frequencies in Australian wild flax and wild flax rust also reflect the process of reciprocal selection. A series of experiments by Burdon (Thompson and Burdon 1992) has shown that particular genotypes of flax rust can develop only on particular genotypes of flax, that flax plants differ in their defenses against particular rust genotypes, and that rusts have demonstrable effects on plant fitness.

To our knowledge, except for brood parasites (Davies and Brooke 1989a, 1989b), there has been no direct demonstration of reciprocal selection between vertebrate hosts and their parasites. The existence of such selection can be inferred from observed evolution (e.g., the rabbit-myxoma virus system; Fenner and Ratcliffe 1965), but this approach does not allow the separate consideration of selection and evolutionary response. In keeping with formal evolutionary theory, we distinguish phenotypic selection, which is a change in the phenotypic distribution of a trait within a generation, from evolutionary response, which is a change in the distribution of a heritable trait across generations (Fisher 1930; Haldane 1954; Arnold and Wade 1984). Although there is broad theoretical agreement that reciprocal selection is a necessary precondition for coevolution, the conditions under which reciprocal selection can operate in natural populations remain unclear (Janzen 1980; Schemske 1983; Rausher 1992; Thompson 1994). In this article, we report the results of controlled experiments designed to test for reciprocal selection on phenotypic components of host defense and parasite escape.

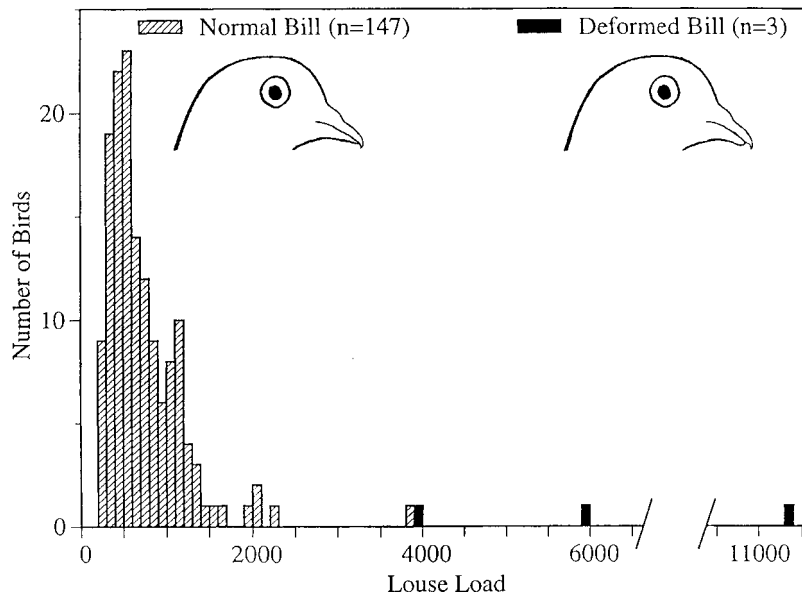


Figure 1: Frequency distribution of lice on adult feral pigeons live-trapped over a 3-wk period (see “Methods”). Lice on the 147 birds with normal bills showed a negative binomial distribution among birds (mean = 631; $K = 1.84$; $\chi^2 = 21.38$, $df = 13$, $P > .05$). The highest louse loads were on birds with minor bill deformities (Mann-Whitney $U = 441$, $P = .003$), such as several millimeters missing from the tip of the lower mandible (illustrated). The frequency of deformities in the study population (2.0%) was similar to that in other populations of pigeons (Clayton 1991) as well as in other species of birds (Pomeroy 1962). The three deformed birds were all in good condition, and one individual was incubating eggs at the time of capture.

Study System

We worked with a simple host-parasite system consisting of feral pigeons, also known as rock doves (*Columba livia*), and two common species of feather lice, *Columbicola columbae* and *Campanulotes bidentatus*. Feather lice are obligate parasites found on virtually all species of birds (Rothschild and Clay 1952; Marshall 1981). The two species we studied are “permanent” parasites that are restricted to the body of the host by appendages specialized for locomotion on feathers (Clayton 1991). Both species of lice feed solely on feathers and dermal debris and complete their entire life cycle on the body of the host; eggs are glued to the feathers with a glandular cement (Nelson and Murray 1971). Transmission among hosts requires physical contact between the feathers of different individual birds, such as that between mated individuals or between parents and their offspring in the nest (Clayton and Tompkins 1994).

Like most birds, feral pigeons use efficient preening to control their lice by disabling or removing them from the plumage (Clayton 1991). Louse loads are usually highest on birds with bill deformities that prevent the full occlusion of the mandibles required for efficient preening (Rothschild and Clay 1952; Ash 1960; Pomeroy 1962; Marshall 1981). In an earlier study (Clayton 1991), feral pi-

geons with minor bill deformities were shown to have significantly higher louse loads than pigeons with normal bills. In the current study, louse loads were also highest on three birds with minor bill deformities (fig. 1). More recently, it has been shown that experimental removal of 1–2 mm from the tip of the upper mandible causes a rapid increase in louse load, similar to the increases seen on naturally deformed birds (D. H. Clayton, unpublished data). When the tip is allowed to grow back, there is a rapid reduction in load to levels equivalent to those on control birds that were never altered.

Although the link between bill morphology and louse load is clear, determining the consequences of increased loads to the fitness of deformed birds is difficult because of the possible confounding effects of such deformities. In this study, we measured the fitness consequences of high loads generated with an experimental manipulation that controls for confounding effects. We tested for an impact of high loads on host survival and reproductive success, the two major components of avian fitness (Price and Boag 1987). We assume that if high loads reduce host fitness, then lice will select for efficient preening and against minor bill deformities.

Lice escape from preening using complex avoidance behavior (Stenram 1956; Clayton 1991). On exposure to

preening, the cigar-shaped wing louse *C. columbae* inserts itself between the barbs of the wing feathers (fig. 14.8 in Clayton 1991). The body louse *C. bidentatus* escapes from preening by dropping from one body feather to another or by burrowing into the thick, downy regions of body feathers. We carried out a reciprocal experiment to test for selective effects of preening on louse body size, which may influence louse escape behavior.

Methods

Effect of Parasite on Host

Fieldwork was conducted on a breeding population of feral pigeons in northeastern Illinois. The population was living under a multisection bridge spanning Rock Creek on Interstate 57, just south of the Manteno exit (41°15.0'N 87°50.3'W). Birds at the bridge were live-trapped over a 3-wk period (May 21–June 11, 1988) by dropping large nets from the railings of different sections of the bridge at night while birds slept at their roosts. Individuals were removed from each netted section with a hand-held net. Each bird's louse load was estimated using regression models that predict total load ($r^2 \geq 0.82$) from timed counts of lice on various body regions (Clayton 1991). Birds were weighed with a Pesola scale, marked with numbered aluminum and colored plastic bands, and released at the bridge within a few hours of capture. We subsequently identified mated pairs of adults by observing which males and females jointly incubated nests; male feral pigeons assist females with incubation (Johnston and Janiga 1995).

We manipulated louse loads as follows. Several weeks after initial capture, birds were recaptured and fitted with bits, which are small (<0.8 g) C-shaped pieces of plated steel that are inserted between the mandibles and crimped slightly in the nostrils to prevent dislodging but without piercing the tissue. Bits create a 1.0–3.0-mm gap between the mandibles that impairs preening, resulting in direct increases in louse load (Clayton 1990, 1991; Booth et al. 1993; Clayton and Tompkins 1995). The increased louse loads of bitted birds mimics, but does not exceed, the increased loads on deformed birds (see "Discussion"). Although bits interfere with preening, they do not interfere with feeding at locations such as our study site, where feral pigeons feed on whole corn and other grain that is easy to pick up despite the mandibular gap created by bits. In an experimental test for possible side effects (Clayton and Tompkins 1995), it was found that bits did not significantly alter the body mass or reproductive success of (parasite-free) feral pigeons; indeed, the reproductive success of bitted birds was slightly higher than that of unbitted birds.

Despite the lack of apparent side effects, we controlled for any undetected side effects by biting every bird in our

study population, with the exception of the three deformed individuals, which were not part of the experiment. Louse increases were prevented on a (randomly chosen) control group of low-load pairs by fumigating them with a 1.0% pyrethrum solution that kills lice with no effect on the bird (Jackson 1985; Clayton and Tompkins 1995). The remaining, high-load, pairs were sham-fumigated with water. All birds were released at the bridge following the biting-fumigation treatment.

We monitored the reproductive success of high- and low-load pairs for 2 mo (August and September). Regular nest checks were made to tabulate the number of eggs hatched by each pair of birds, as well as the number of nestlings that subsequently fledged. Nestlings were marked with numbered aluminum bands at approximately 10 d of age. Fledging success was analyzed contingent on the number of eggs hatched in a given nest.

The effectiveness of biting/fumigation was assessed by recapturing birds in September, 3 mo following initial treatment, and estimating their louse loads. An ordinal scoring system reported by Booth et al. (1993) was used to estimate loads because it minimized handling time and thus the amount of time birds had to be kept away from their nests. For each bird, the number of lice on the undersurface of one wing was scored visually as 1 (low), 2 (moderate), or 3 (high). The number of lice on the rump was scored in the same way. The wing and rump scores were then added together to provide an overall score for each bird ranging from 2 to 6.

To monitor body mass and feather damage, birds were weighed and feather samples taken as described in figure 2. Feather samples were weighed in the laboratory to the nearest 0.01 mg on an analytical balance. Low-load birds were refumigated to kill any lice on reinfested individuals, and high-load birds were again sham-fumigated.

We compared the long-term survival of high- and low-load birds by recapturing all individuals still living under the bridge 1 yr following the start of the experiment (June 1989). The ownership and status of nests belonging to recaptured birds were also determined as a spot check of reproductive activity by high- versus low-load adult pairs still living under the bridge. Bits were removed from all birds before final release.

Effect of Host on Parasite

The reciprocal experiment was conducted using captive pigeons bred from individuals captured about 100 mi from our field site (Clayton 1991). Since the species of lice in our study spend all of their time on the body of the host, the captive birds represented natural habitat for the parasites. Eighteen birds were bitted several months before the experiment to boost their louse loads. The birds were

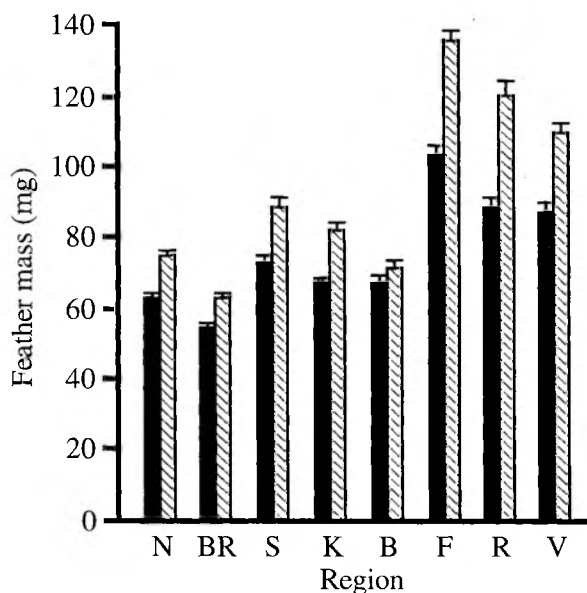


Figure 2: Mean (\pm SEM) mass of feathers from 58 high-load birds (solid bars) and 56 low-load birds (striped bars). The value for each bird is the combined mass of the 10 longest feathers of a clump plucked from each body region: N = nape, BR = breast, S = side (under wing), K = adjacent to keel, B = back, F = flank, R = rump, and V = ventral caudal tract (Booth et al. 1993, fig. 1). Wing and tail feathers were not sampled because they are not consumed by lice (Nelson and Murray 1971).

randomly isolated in 18 cages arranged in pairs, with one experimental and one control to a pair. The cages were kept in a barn under ambient conditions of temperature and humidity. Birds were given ad lib. grit, water, and a commercial grain mixture (Kaytee Breeder and Conditioner Pigeon Feed 6). Cages were separated to guard against contact between any feathers protruding through the cage mesh, thus preventing transmission of lice between birds.

At time 1 (T1), 100 adult lice of each species were collected from each bird and preserved in 70% alcohol. Roughly equal numbers of lice were collected from all host body regions (fig. 1 in Booth et al. 1993) by squirting them with a small amount of alcohol, thus preventing even the most agile lice from escaping through the plumage (the species in this study do not move all that quickly in any case). Dead lice were immediately removed from the plumage with forceps. The alcohol dried quickly, leaving no residual effects. Immediately after the T1 collection, the bits were removed from the nine experimental birds to reinstate preening; bits were left in place on the nine control birds.

At time 2 (T2), all lice remaining on each bird were collected and preserved, being sure to examine every body region. An average of 26.4 d (SD = 1.04) elapsed between

the T1 and T2 samples, maximizing the episode of selection without exceeding the time to first reproduction of the lice (27 d on average; Marshall 1981). Phenotypic selection is best measured within a generation to avoid confounding it with the evolutionary response to selection (Manly 1985; Brodie et al. 1995).

For body size data, five adult male and female lice of each species from each bird were randomly selected from the T1 samples and mounted on microscope slides. Five adult male and female lice of each species from each bird were also randomly selected from the T2 samples and mounted on slides. Fewer than five lice of each sex remained on a few of the birds at T2, in which case all lice were mounted. An ocular micrometer on a phase-contrast microscope was used to measure the body lengths of slide-mounted lice (see fig. 3 in Clayton and Price 1984 for landmarks). Only adult lice were measured because adults do not change in body size throughout this final stage of the life cycle. Measurements were made by a researcher who was blind to host treatment. The body length measurement was highly repeatable for both species of lice (*Campanulotes*, $r = 0.88$; *Columbicola*, $r = 0.98$; $P = .0001$ for both; $N = 25$ males of each). Repeatabilities were calculated from two measurements of each specimen on different days (Lessells and Boag 1987).

Selection on body length was investigated separately for each species of louse and bit treatment. Selection was also investigated separately for the two sexes since female feather lice are typically 15% larger than males (Marshall 1981). Mean body sizes at T1 and T2 were compared using a two-way ANOVA, with time, sex, and time \times sex interaction as factors. Analyses were performed using JMP version 3.1 (SAS Institute 1985).

Results

Effect of Parasite on Host

High-load birds (bitted and sham fumigated) had significantly more lice than low-load birds (bitted and fumigated) 3 mo following treatment. Sixty-five high-load birds had a mean louse score of 4.2 (SD = 1.3), compared with a score of 2.8 (SD = 1.4) for 62 low-load birds (Mann-Whitney $U = 945$, $P = .0001$).

The feathers of high-load birds weighed 19% less, on average, than those of low-load birds 3 mo after treatment (fig. 2). The overall mean feather mass of 58 high-load birds was 0.60 mg (SD = 0.08), compared with a mean of 0.74 mg (SD = 0.09) for 56 low-load birds. A repeated-measures ANOVA of the feather mass data revealed highly significant effects of treatment ($F = 76.61$, $df = 1, 112$, $P < .001$), body region ($F = 280.55$, $df = 7, 784$, $P < .001$), and treatment \times region interaction ($F = 16.80$, $df =$

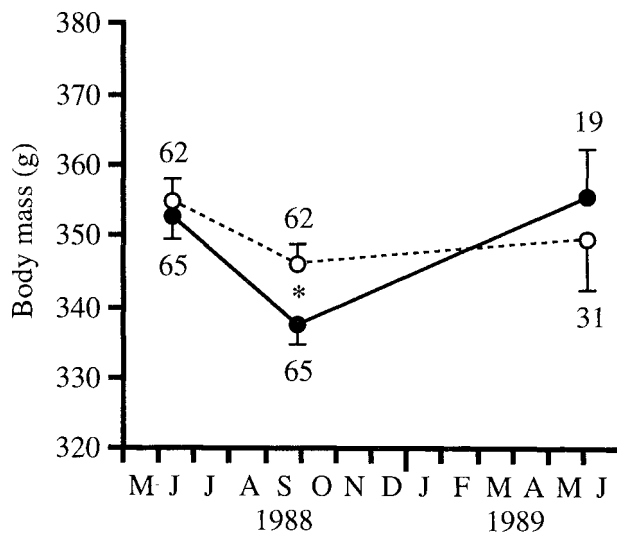


Figure 3: Mean (\pm SEM) body mass of high-load birds (solid circles) and low-load birds (open circles) over the course of the study. Numbers above and below the error bars are sample sizes. Only birds captured at the start of the study, and again 3 mo later, are included. The June 1989 data are for all birds recaptured 1 yr following treatment. Significant differences ($P < .05$) are indicated by an asterisk.

7,784, $P < .001$). Feather damage was not ameliorated by the annual molt, which started 1 mo following treatment and required several months to complete (D. H. Clayton, unpublished data). Spot checks showed that large numbers of lice, with accompanying feather damage, were present on high-load birds throughout the autumn.

High-load birds weighed 2.4% less, on average, than low-load birds 3 mo following treatment (fig. 3). The mean body mass of high-load birds was 337.5 g (SD = 25.6), compared with a mean of 345.8 g (SD = 22.8) for low-load birds ($t = 1.92$, $df = 125$, one-tailed $P = .029$). One year after treatment (June 1989) there was no longer a significant difference in the body masses of high- and low-load birds (fig. 3). The mean body mass of high-load birds was 355.8 g (SD = 31.2), compared with a mean of 349.5 g (SD = 39.0) for low-load birds ($t = 0.63$, $df = 44$, one-tailed $P = .73$).

Louse load had no significant effect on the hatching success of eggs incubated by high-load birds; 14 high-load pairs hatched a mean of 0.93 eggs (SD = 0.83), compared with a mean of 0.85 eggs (SD = 0.99) hatched by 13 low-load pairs ($U = 83$, $P = .70$). There was also no significant effect of louse load on the fledging success of hatched birds; nine of 13 (69%) young in high-load nests fledged, compared with eight of 11 (73%) young in low-load nests (Fisher exact $P = .60$). Finally, there was no detectable long-term effect of louse load on nesting activity; seven

of seven high-load nests contained fresh eggs or young, compared with five of five low-load nests (Fisher exact $P = .42$) 1 yr after the start of the experiment.

There was no effect of louse load on short-term recapture rates; 65 of 80 (81%) high-load birds were recaptured in September, compared with 63 of 74 (85%) low-load birds ($\chi^2 = 0.17$, $P = .68$). In contrast, there was a significant long-term effect of louse load on host recapture (fig. 4), with 19 of 65 (29%) high-load birds recaptured after a year, compared with 31 of 62 (50%) low-load birds.

Effect of Host on Parasite

Preening selected for smaller body size in both species of lice (table 1). There was no significant effect of time on the body length of lice on control birds bitten throughout the experiment. In contrast, there was a significant effect of time on the body length of both species of lice on experimental birds that had their bits removed after T1. As expected, there were also significant differences in the body sizes of male and female lice within each species, treatment, and time. However, there was no sex \times time interaction within species or treatment, indicating that male and female lice experienced similar selection, despite the fact that female lice are 15% larger than males.

A graphical comparison of adjusted mean body lengths (fig. 5) shows the reduction in mean length of both species of lice on experimental birds. *Campanulotes* had a mean reduction of 10 μ on experimentals, compared with only 5 μ on controls. *Columbicola* had a mean reduction of 32 μ on experimentals, compared with only 13 μ on controls (fig. 5).

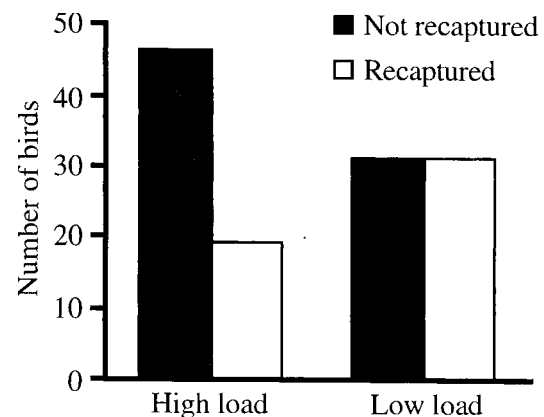


Figure 4: Recapture rates of high- and low-load pigeons. The number of birds recaptured in June 1989 was compared with the number of birds treated the previous June (and recaptured and retreated in September 1988). The recapture rate of high-load birds was significantly less than that of low-load birds ($\chi^2 = 5.73$, $P < .017$).

Table 1: ANOVA for body lengths of two species of lice

Source and species	df	F ratio	Significance
Bitted throughout:			
<i>Campanulotes:</i>			
Model	3	1,248.97	.0001
Error	169		
Time	1	1.05	NS
Sex	1	3,736.60	.0001
Time × sex	1	.33	NS
<i>Columbicola:</i>			
Model	3	575.70	.0001
Error	174		
Time	1	1.99	NS
Sex	1	1,724.85	.0001
Time × sex	1	.00	NS
Bits removed after T1:			
<i>Campanulotes:</i>			
Model	3	1,152.68	.0001
Error	162		
Time	1	3.9058	.05
Sex	1	3,432.94	.0001
Time × sex	1	.11	NS
<i>Columbicola:</i>			
Model	3	555.10	.0001
Error	172		
Time	1	10.43	.002
Sex	1	1,647.95	.0001
Time × sex	1	.10	NS

Note: T1 = time 1.

Discussion

Our results confirm that hosts with impaired preening, owing to slight bill deformities, have higher feather louse loads than hosts with normal bills. We also show for the first time that high feather louse loads reduce the survival of hosts as a result of the extensive feather damage they cause. Together, these results suggest that lice will exert selection for efficient preening ability. Our reciprocal experiment demonstrates that preening with a normal bill, in turn, selects for small body size in lice, which may facilitate their escape from preening. The results of this study verify a crucial element of coevolutionary theory by identifying likely targets of reciprocal phenotypic selection between host and parasite.

Effect of Parasite on Host

Tests for effects of parasites on host fitness are often complicated by the aggregated distribution of parasites among hosts in natural populations (Grenfell and Dobson 1995). When parasites are aggregated, relatively few host individuals have high loads. This was the case for the parasites in this study, which showed a negative binomial distri-

bution among hosts (fig. 1). Contrary to the early reasoning of some evolutionary ecologists (Lack 1954), the infrequency of high loads is not evidence for parasites having little impact on host fitness. Rather, aggregation may be a direct consequence of the evolution of efficient defense by hosts in response to potent selection by parasites. The permanence of lice, which require direct contact between host individuals for transmission, may also con-

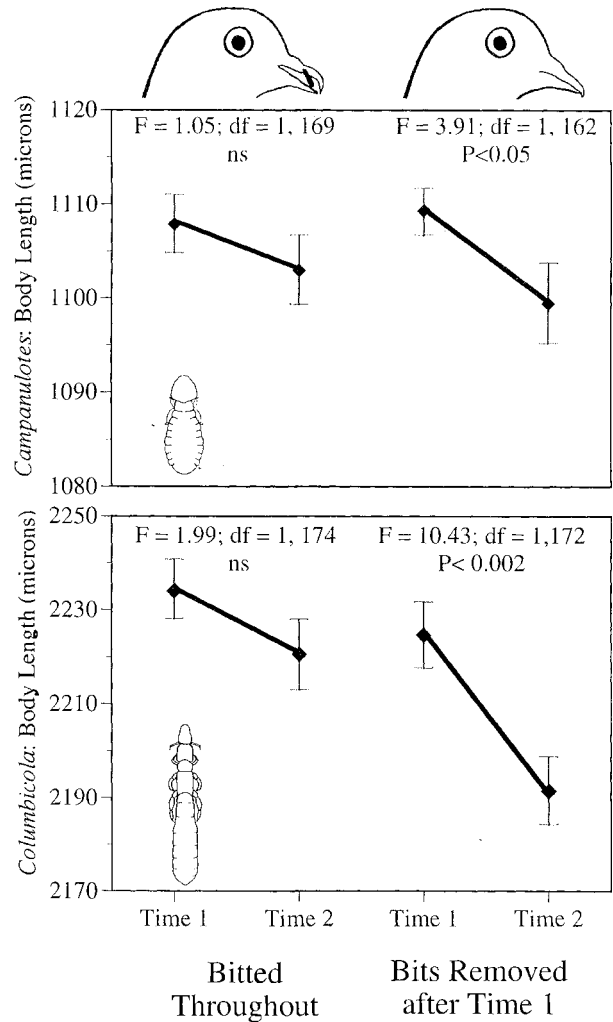


Figure 5: Selective effect of preening on the morphology of two species of pigeon lice *Campanulotes bidentatus* and *Columbicola columbae* (drawn to the same scale, with sexes arbitrarily set equal to male mean for comparison). Preening-imposed selection was relaxed for several louse generations by biting all birds before the experiment. Samples of adult lice were collected at T1, then the preening ability of experimental birds was restored by removing their bits. Postselection samples of adult lice were collected at T2. Body lengths (mean ± 1 SEM) of both species decreased significantly on birds with restored preening but did not change significantly on birds that wore bits throughout the trial.

tribute to the aggregated distribution by restricting dispersal of lice from heavily infested hosts.

Aggregated distributions present an empirical dilemma. It is difficult to study the effects of aggregated parasites using a correlational approach because finding heavily infested hosts requires very large sample sizes. A more powerful approach is to use experimental manipulations to convert the aggregated distribution into a bimodal distribution of high- and low-load hosts, which also helps control for unknown covariates of parasite load and host fitness. We adopted such an approach in the form of bits, which simulated the inefficient preening of birds with deformities. As in previous studies (Clayton 1990, 1991; Booth et al. 1993; Clayton and Tompkins 1995), the combination of biting and fumigation created high- and low-load groups of birds. Although the distribution of lice on nonfumigated (experimental) birds remained aggregated, most of these birds had louse loads in excess of those on fumigated (control) birds.

For natural conditions to be properly simulated, experimental manipulations of parasite load should not exceed the range of natural variation in load. We estimated louse load over the course of our experiment using an ordinal scoring system that minimized handling time. The use of ordinal scoring precluded a quantitative comparison of lice on experimental birds with the louse loads of naturally deformed birds (fig. 1). However, previous work shows that biting does not generate unnaturally high loads. For example, in an earlier study (Clayton and Tompkins 1995), 86 feral pigeons were bitten and kept under densely populated conditions. Although density causes increases in louse load (Clayton 1991), none of the bitten birds in the captive flock had more than 11,000 lice (cf. fig. 1). Pigeons with similarly high louse loads have also been captured at other field sites (D. H. Clayton, unpublished data).

Experimental high-load birds had significantly more feather damage than low-load birds within 3 mo of treatment (fig. 2). The reduction in feather mass on our high-load birds was similar in extent to that shown in other studies (Clayton 1990, 1991; Booth et al. 1993). Our study revealed a 2.4% mean reduction in host body mass within 3 mo (fig. 3), which compares favorably with the results of another biting experiment at a bridge near our site (Booth et al. 1993). The earlier experiment showed a 3.6% reduction in mean body mass of bitten birds after 5 mo and a 6.8% reduction after 9 mo. Body mass and feather damage were significantly correlated among the birds in our study ($r = 0.33$, $P = .0003$, $N = 114$).

Significantly fewer high load birds were recaptured 1 yr following initial treatment (fig. 4). Birds not recaptured were assumed to have died over the winter. The 50% mortality of low-load birds in our study compares favorably

with data from other populations of feral pigeons, in which mortality rates as high as 80% have been estimated (Morton and Clarke 1968). Cold weather is considered the principal source of mortality for adult feral pigeons (Johnston and Janiga 1995). Birds in our experiment were subjected to extremely cold weather, with a mean winter (December 1988–March 1989) temperature of -18.5°C and a low of -33.5°C (National Oceanic and Atmosphere Administration [NOAA] 1988, 1989).

Because we did not recover the bodies of birds presumed to have died, we could not distinguish mortality from dispersal with certainty. However, it is unlikely that the lower recapture rate of high-load birds was because of dispersal. Opportunities for local dispersal were severely limited because the bridge at our study was surrounded by expanses of open farmland; feral pigeons depend on man-made structures for roosting (Johnston and Janiga 1995). Long-distance dispersal is equally unlikely because adult feral pigeons are sedentary, not normally dispersing more than a few hundred meters (Johnston 1992; Johnston and Janiga 1995). No bird from our study population was ever captured at a bridge 5 mi down the highway, where we were concurrently studying another large population of feral pigeons.

The proximal reason for the effect of lice on host fitness presumably had to do with the energetic cost of feather damage (fig. 2). In an earlier biting experiment, Booth et al. (1993) showed that feather damage from lice causes an increase in whole-body thermal conductance in response to the reduced insulative effectiveness of the plumage. Birds with damaged feathers maintained a constant body temperature, despite this increase in thermal conductance, by elevating metabolic rates 8.5%, on average. These elevated metabolic rates were maintained by drawing on stored reserves, leading to a gradual reduction in body mass similar to the one in our study (fig. 3). We suspect that the reduced recapture rates of high-load birds (fig. 4) reflects mortality owing to the energetic cost of reduced insulation.

The similar recapture rates we measured for high- and low-load birds in September is consistent with the energetics hypothesis just described. During the 3 mo following initial treatment (July–September 1988), birds were not exposed to very cold weather (mean temperature during this period was 23.5°C ; NOAA 1988, 1989). Although recapture rates were similar in September, the body mass of high-load birds was significantly less than that of low-load birds (fig. 3). This result suggests that pigeons were experiencing energetic stress even during the warm weather, consistent with data showing such stress in high-load birds at relatively high ambient temperatures (Booth et al. 1993). Although warm weather stress apparently caused a reduc-

tion in the body mass of high-load birds, the stress was not sufficient to cause mortality within the first 3 mo.

One year following initial treatment, there was no longer a significant difference in the body mass of high- and low-load birds (fig. 3). We interpret this to be the result of selection against high-load birds with low initial body mass. Since birds were not captured and weighed over the 9-mo period between September 1988 and June 1989, it is not possible to test this hypothesis in a rigorous way. However, a comparison of the September body mass of high-load birds recaptured the following June with the body mass of high-load birds not recaptured reveals a trend toward higher September body mass in recaptured individuals.

We detected no effect of lice on the reproductive success of high-load birds during a 2-mo period (August and September 1988). Given that feather damage from lice accumulates gradually, our study may well have been too brief to have detected potential effects of lice on host reproduction. There was also no evidence that louse load affected reproductive activity 1 yr following treatment, but this comparison was a spot check of a small number of breeding pairs. Similarly, in a longer-term experiment lasting several months (Clayton and Tompkins 1995), no effect of lice on the number of eggs laid, hatching success, nestling growth rates, fledging success, postfledging body mass, or fledgling survival was found in a captive flock of pigeons bred from birds captured near our study site. A longer-term field experiment is needed to test for chronic effects of lice on host reproductive success, particularly since feral pigeons breed 9 mo a year, including during early spring and late autumn cold weather (Johnston and Janiga 1995).

Feather lice have long been thought to have little or no effect on birds (Rothschild and Clay 1952; Ash 1960; Marshall 1981). However, the results of our study show that feather lice can have a significant impact on host survival. The effect of feather lice is chronic and thus difficult to demonstrate. It is unlikely that we would have detected an effect on host fitness had we not manipulated louse loads over the course of a long-term experiment.

Effect of Host on Parasite

The selective effect of preening for small body size presumably followed an increase in size during the preexperimental period, when all birds wore bits for several months (i.e., under relaxed selection before T1; see "Methods"). We were unable to test for an increase in body size because we did not collect lice before biting. Diluting the already small populations of lice on prebitten birds, through collecting, would have increased the probability of stochastic extinction on some birds during the course

of the experiment. Although we did not test for an increase in body size, such an increase seems feasible in light of the positive correlation often shown between body size and fecundity in invertebrates (Sibly and Calow 1986). A comparison of the body size of lice in our experiment with that of lice on unmanipulated birds in the field is needed.

Whatever the initial size of lice, our study reveals that preening can select for small body size in lice (fig. 5). *Campanulotes* on experimental birds showed a mean reduction in body length of 10 μ , and *Columbicola* showed a mean reduction of 32 μ . Although these changes may seem small, it has recently been shown (Tompkins and Clayton 1999) that survival of host-specific lice transferred between species of birds is governed by the dimensions of tiny feather barbs. Species in the genus *Dennyus*, which parasitize swifts, cannot survive when moved to "foreign" hosts with mean barb diameters differing from those on the "usual" host by more than 2.5 μ . At this scale, the size reductions in lice shown in the current study were substantial.

Preening may have selected for small body size because smaller lice are better able to escape. *Campanulotes* spends most of its time on the abdomen and escapes by dropping from one body feather to another or by running through the downy regions of body feathers (Clayton 1991). Small size may improve the ability of *Campanulotes* to maneuver through the downy matrix of the feather. *Columbicola* spends most of its time on the underside of the wings and escapes by inserting itself between the rigid barbs of flight feathers (Clayton 1991). Small size could improve the ability of this louse to fit between feather barbs. *Columbicola* body width, which may be a determinant of interbarb fit, is highly correlated with body length (D. H. Clayton, unpublished data).

Interestingly, the selective effect of preening did not differ between male and female lice, despite the fact that females are 15% larger than males. This result suggests that the two sexes avoid preening in different ways by using different microhabitats on the host. For example, male *Columbicola* may prefer the more delicate secondary flight feathers, while females prefer coarse primary feathers. Although data do not yet exist with which to test this differential microhabitat use hypothesis, Nelson and Murray (1971) published data suggesting that male and female pigeon lice differ somewhat in the major body regions on which they are found.

Reciprocal selection is the unique mechanism behind coevolution (Janzen 1980; Futuyma and Slatkin 1983; Thompson 1994). Although work on coevolution has come a long way since Ehrlich and Raven's (1964) seminal article, much of this work has focused predominantly on one side of the coevolutionary coin. The reason for this is that it is often logistically difficult to study interacting

partners equally. The overriding goal of this study was to perform a balanced pair of experiments to identify specific targets of reciprocal selection between host and parasite phenotypes. Additional work is needed to characterize how reciprocal selection actually occurs in nature, as well as the dynamics of coevolutionary responses to reciprocal selection.

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Literature Cited

- Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: applications. *Evolution* 38:720–734.
- Ash, J. S. 1960. A study of the Mallophaga of birds with particular reference to their ecology. *Ibis* 102:93–110.
- Booth, D. T., D. H. Clayton, and B. A. Block. 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proceedings of the Royal Society of London B, Biological Sciences* 253:125–129.
- Brodie, E. D., III, A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends in Ecology & Evolution* 10:313–318.
- Clayton, D. H. 1990. Mate choice in experimentally parasitized rock doves: lousy males lose. *American Zoologist* 30:251–262.
- . 1991. Coevolution of avian grooming and ectoparasite avoidance. Pages 258–289 in J. E. Loya and M. Zuk, eds. *Bird-parasite interactions: ecology, evolution, and behaviour*. Oxford University Press, Oxford.
- Clayton, D. H., and R. D. Price. 1984. Taxonomy of the *Strigiphilus cursitans* species group (Ischnocera: Philoptera: Philoptera), parasites of owls. *Annals of the Entomological Society of America* 77:340–363.
- Clayton, D. H., and D. M. Tompkins. 1994. Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London B, Biological Sciences* 256:211–217.
- . 1995. Comparative effects of mites and lice on the reproductive success of rock doves (*Columba livia*). *Parasitology* 110:195–206.
- Davies, N. B., and M. de L. Brooke. 1989a. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *Journal of Animal Ecology* 58:207–224.
- . 1989b. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *Journal of Animal Ecology* 58:225–236.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586–608.
- Fenner, F., and F. N. Ratcliffe. 1965. *Myxomatosis*. Cambridge University Press, Cambridge.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford University Press, Oxford.
- Futuyma, D. J., and M. Slatkin. 1983. *Coevolution*. Sinauer, Sunderland, Mass.
- Grenfell, B. T., and A. P. Dobson. 1995. *Ecology of infectious diseases in natural populations*. Cambridge University Press, Cambridge.
- Haldane, J. B. S. 1954. The measurement of natural selection. *Proceedings of the Ninth International Congress of Genetics 1(Caryologica suppl.):480–487*.
- Jackson, J. A. 1985. On the control of parasites in nest boxes and the use of pesticides near birds. *Sialia* 7:17–25.
- Janzen, D. H. 1980. When is it coevolution? *Evolution* 34:611–612.
- Johnston, R. F. 1992. Rock dove. Pages 1–16 in A. Poole, P. Stettenheim, and F. Gill, eds. *The birds of North America*. American Ornithologists' Union, Washington, D.C.
- Johnston, R. F., and M. Janiga. 1995. *Feral pigeons*. Oxford University Press, New York.
- Lack, D. 1954. *The natural regulation of animal numbers*. Oxford University Press, London.
- Lessells, C. M., and P. T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121.
- Manly, B. F. J. 1985. *The statistics of natural selection on animal populations*. Chapman & Hall, London.
- Marshall, A. G. 1981. *The ecology of ectoparasitic insects*. Academic Press, London.
- Murton, R. K., and S. P. Clarke. 1968. Breeding biology of rock doves. *British Birds* 61:429–448.

- National Oceanic and Atmospheric Administration (NOAA). 1988. Climatological data annual summary for Illinois. Vol. 92. NOAA Environmental Data and Information Service, National Climactic Center, Asheville, N.C.
- National Oceanic and Atmospheric Administration (NOAA). 1989. Climatological data annual summary for Illinois. Vol. 93. NOAA Environmental Data and Information Service, National Climactic Center, Asheville, N.C.
- Nelson, B. C., and M. D. Murray. 1971. The distribution of Mallophaga on the domestic pigeon (*Columba livia*). *International Journal of Parasitology* 1:21–29.
- Pomeroy, D. E. 1962. Birds with abnormal bills. *British Birds* 55:49–72.
- Price, T. D., and P. T. Boag. 1987. Selection in natural populations of birds. Pages 257–288 *in* F. Cooke and P. A. Buckley, eds. *Avian genetics*. Academic Press, London.
- Rausher, M. D. 1992. Natural selection and the evolution of plant-insect interactions. Pages 20–88 *in* B. D. Roitberg and M. B. Isman, eds. *Insect chemical ecology: an evolutionary approach*. Chapman & Hall, New York.
- Rothschild, M., and T. Clay. 1952. Fleas, flukes and cuckoos. Collins, London.
- SAS Institute. 1985. JMP user's guide. SAS Institute, Cary, N.C.
- Schemske, D. W. 1983. Limits to specialization and coevolution in plant-animal mutualisms. Pages 67–110 *in* M. H. Nitecki, ed. *Coevolution*. University of Chicago Press, Chicago.
- Sibly, R. M., and P. Calow. 1986. *Physiological ecology of animals*. Blackwell Scientific, Oxford.
- Stenram, H. 1956. The ecology of *Columbicola columbae* L. (Mallophaga). *Opuscula Entomologica* 21:170–190.
- Thompson, J. N. 1994. *The coevolutionary process*. University of Chicago Press, Chicago.
- Thompson, J. N., and J. J. Burdon. 1992. Gene-for-gene coevolution between plants and parasites. *Nature (London)* 360:121–125.
- Tompkins, D. M., and D. H. Clayton. 1999. Host resources govern the specificity of swiftlet lice: size matters. *Journal of Animal Ecology* 68:489–500.

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