TANGLED TREES

Phylogeny, Cospeciation, and Coevolution

EDITED BY Roderic D. M. Page

II

COEVOLUTIONARY HISTORY OF ECOLOGICAL REPLICATES: COMPARING PHYLOGENIES OF WING AND BODY LICE TO COLUMBIFORM HOSTS

Kevin P. Johnson and Dale H. Clayton

THE UNIVERSITY OF CHICAGO PRESS
Chicago and London 2003

II

COEVOLUTIONARY HISTORY OF ECOLOGICAL REPLICATES: COMPARING PHYLOGENIES OF WING AND BODY LICE TO COLUMBIFORM HOSTS

Kevin P. Johnson and Dale H. Clayton

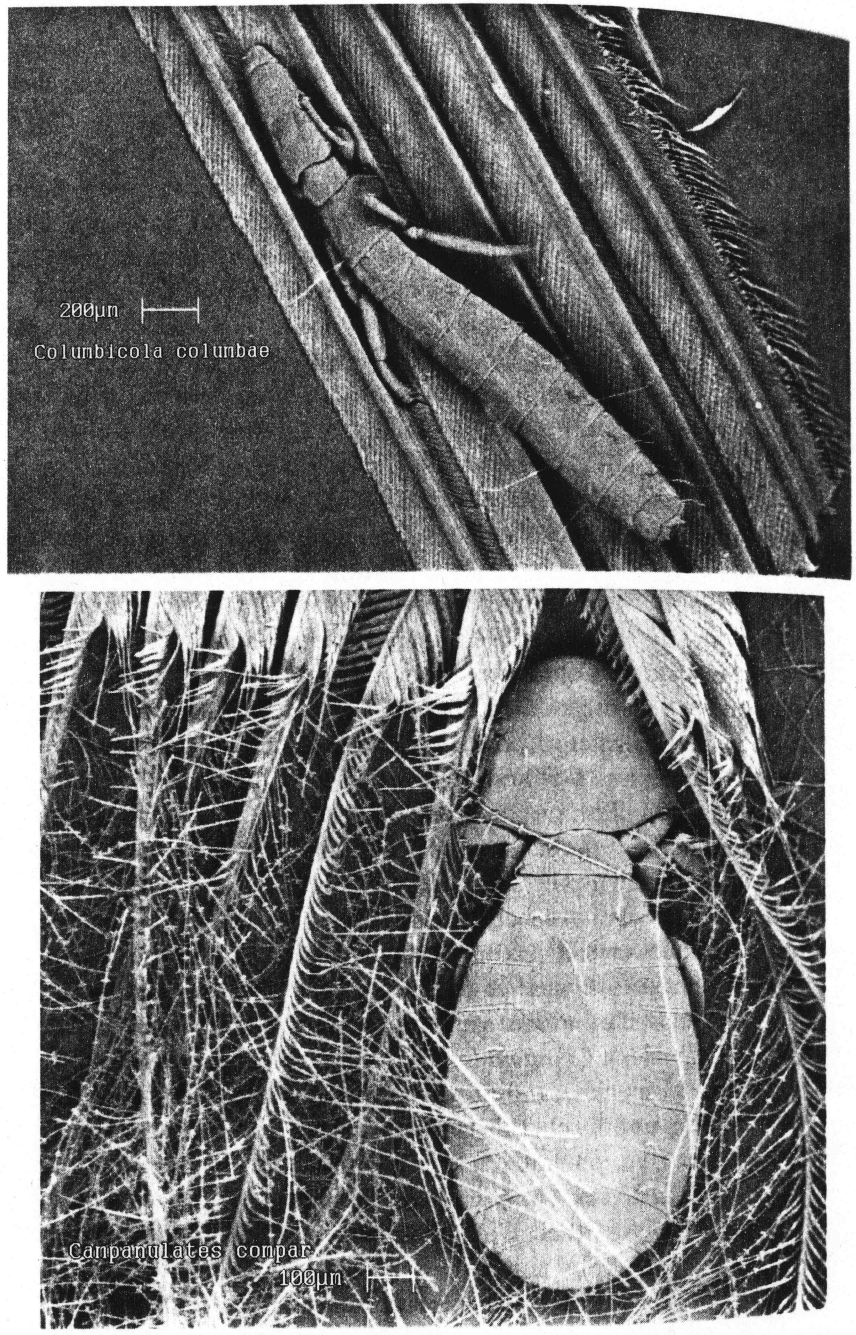
Phylogenies depict the history of speciation for groups of organisms. Comparing the phylogenies of interacting groups can reveal instances of tandem speciation, or "cospeciation" (Brooks and McLennan, 1991; Hoberg et al., 1997; Paterson and Gray, 1997). Understanding the conditions under which cospeciation takes place is a challenging task. In the case of hosts and their parasites, cospeciation occurs when isolation of host populations also isolates the parasites on those hosts. Patterns of cospeciation can break down owing to dispersal of parasites among host populations, sympatric speciation of parasites on a single host population, or extinction of parasites on a host population (Page and Charleston, 1998). All else being equal, ecologically similar parasites living on the same host should respond to isolation of host populations in the same way, yielding similar coevolutionary histories. In this chapter we compare cospeciation events in two such "replicate" groups of lice living on the same hosts. If forces promoting speciation, such as host speciation, act on these parasites in similar ways, then we would expect cospeciation events to be correlated between these parasite groups. On the other hand, if the parasites respond to isolation differently, then cospeciation events should be independent in the two groups.

We focus on two groups of Ischnoceran feather lice (Insecta: Phthiraptera), both of which are found on pigeons and doves (Aves: Columbiformes). Feather lice are permanent parasites that are restricted to the body of the host by appendages specialized for locomotion on feathers (Clayton, 1991). They complete their entire life cycle on the body of the host, where they feed on feathers and dermal debris. Transmission among hosts usually occurs through physical contact between the feathers of different individual birds, such as that between mated individuals or between parents and their offspring in the nest (Marshall, 1981).

Columbiform feather lice are of two distinct morphological types: wing lice and body lice. Wing lice are long and slender (fig. 11.1, top) and lay their eggs on the wing and tail feathers of their host. Their shape is an adaptation for inserting between feather barbs, which helps them (1) adhere to the host during flight, and (2) avoid being removed by the host when it preens (Clayton, 1991). Body lice, which have a more rounded shape (fig. 11.1, bottom), live primarily on the host's abdominal feathers, where they escape from preening by burrowing in the downy portions of these feathers (Clayton, 1991). Despite their differences in body form and mechanisms of escape, Columbiform wing and body lice are ecologically very similar. Both feed on abdominal contour feathers (Nelson and Murray, 1971) and have similar effects on host fitness (Booth et al., 1993; Clayton and Tompkins, 1995; Clayton et al., 1999). Both are directly transmitted to nestlings (Clayton and Tompkins, 1994); however, both have also been recorded "hitchhiking" phoretically on hippoboscid flies (Couch, 1962).

A historical comparison of ecological replicates would be compromised if their phylogenies were intertwined. In addition to being ecologically similar, Columbiform wing and body lice are phylogenetically independent. A phylogeny based on DNA sequences for a number of Ischnoceran genera indicates that Columbiform wing and body lice are each monophyletic and are distantly related within the Ischnocera (Cruickshank et al., 2001). Body lice are sister to several genera of Galliform lice. The body lice on Columbiformes and Galliformes together form the family Goniodidae (Smith, 2000). Wing lice, on the other hand, appear to be a basal lineage of the Ischnocera, but the sister taxon to Columbiform wing lice currently cannot be identified with certainty (Cruickshank et al., 2001). Most species of pigeons and doves have both wing and body lice (Hopkins and Clay, 1952; Price, unpub. checklist). Based on morphology, wing lice are classified in two genera: *Columbicola* (67 species) and *Turturicola* (8 species). Body lice are classified in five genera containing 141 described species: *Auricotes* (45 species), *Campanulotes* (12 species), *Coloceras* (58 species), *Kodocephalon* (3 species), and *Physconelloides* (23 species) (Hopkins and Clay, 1952; Price, unpub. checklist).

In this chapter we compare phylogenies of wing lice (*Columbicola* only) and body lice (*Auricotes, Campanulotes, Coloceras,* and *Physconelloides*) with the phylogeny of their Columbiform hosts. These phylogenies are based on both nuclear and mitochondrial DNA sequences. We infer nodes in the tree that show apparent cospeciation between dove hosts and louse parasites. A novel aspect of our study is that we examine two parasite groups living on the same hosts. If multiple groups of parasites respond



to host speciation in similar ways, we would expect congruence between parasite phylogenies, as well as between host and parasite phylogenies. Although wing and body lice are similar ecologically, detailed differences between the two may influence cospeciation patterns. If one group of parasites can disperse more easily than the other, one might expect that speciation events in the host would be less of a barrier to gene flow among the populations of that group. Differences in survival ability on multiple host species could also create differences in the pattern of cospeciation. Moreover, chance events might play a role in breaking down congruence between the phylogenies of two groups of parasites on the same group of hosts. For example, stochastic extinction of parasites might cause the phylogenies of two groups of parasites on the same group of hosts to show little similarity. In our study, we evaluate the extent to which cospeciation events are common to both wing and body lice, and whether the coincidence of such events is more frequent than expected by chance.

Methods

Samples and DNA Sequencing

We extracted DNA from frozen samples of host tissue using the protocol described by Johnson and Clayton (2000). We included 19 species of Columbiformes in this study with representatives of two divergent subspecies of one of the species: *Leptotila verreauxi* (table 11.1). Lice were sampled from wild hosts using techniques described in Clayton and Walther (1997). Lice were either frozen at −70°C or stored at −20°C in 95% ethanol. For each louse we carefully removed the head from the body and extracted DNA from both using a Qiagen tissue kit. After the DNA extraction procedure, the head and body of the louse were reassembled as a voucher specimen mounted in balsam on a microslide, which was used for identification. PCR and sequencing was done as described by Johnson and Clayton (2000).

For hosts, we sequenced 2,589 base pairs, including portions of the mitochondrial genes cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) and the nuclear gene β-fibrinogen intron 7 (FIB7) gene. See Johnson and Clayton (2000) for cyt *b* and FIB7 primers, and Hafner et al. (1994) for COI primers (Genbank accession numbers AF182649, AF182650, AF182653, AF182658, AF182661, AF182663, AF182668, AF182670, AF182673, AF182682, AF182686, AF182686, AF182691, AF182697, AF182701,

FIGURE 11.1. (*Facing page*) SEMs of feather lice from the Rock Dove (*Columba livia*). Top, *Columbicola columbae*, a wing louse; bottom, *Campanulotes* (*bidentatus*) *compar*, a body louse.

TABLE 11.1 Host and parasite taxa included in study

Host	Wing Louse	Body Louse
Columbina inca	*Columbicola passerinae* 1	*Physconelloides eurysema* 1
Columbina passerina	*Columbicola passerinae* 1	*Physconelloides eurysema* 2
		Physconelloides eurysema 3
Claravis pretiosa	*Columbicola passerinae* 2	*Physconelloides eurysema* 2
Phapitreron amethystina	*Columbicola exilicornis*	*Coloceras clypeatum*
Phapitreron leucotis	*Columbicola veigasimoni*	*Coloceras* n. sp. 1
Ptilinopus occipitalis	*Columbicola xavieri*	*Auricotes rotundus*
Columba speciosa	*Columbicola adamsi*	*Physconelloides spenceri*
Columba plumbea	*Columbicola adamsi*	*Physconelloides anolaimae*
Streptopelia senegalensis	*Columbicola theresae*	*Coloceras* n. sp. 2
Streptopelila capicola	*Columbicola theresae*	*Coloceras* n. sp. 2
Columba livia	*Columbicola columbae* 1	*Campanulotes compar*
Columba guinea	*Columbicola columbae* 2	*Coloceras savoi*
Zenaida asiatica	*Columbicola macrourae* 2	*Physconelloides wisemani*
Zenaida macroura	*Columbicola macrourae* 3	*Physconelloides zenaidurae*
	Columbicola baculoides	
Zenaida galapagoensis	*Columbicola macrourae* 4	*Physconelloides galapagensis*
Geotrygon montana	*Columbicola macrourae* 1	*Physconelloides cubanus*
Leptotila plumbeiceps	*Columbicola macrourae* 1	*Physconelloides ceratoceps* 1
	Columbicola gracilicapitis	*Physconelloides ceratoceps* 2
		Physconelloides ceratoceps 3
Leptotila jamaicensis	*Columbicola gracilicapitis*	*Physconelloides ceratoceps* 1
Leptotila verreauxi angelica	*Columbicola macrourae* 2	*Physconelloides ceratoceps* 3
	Columbicola macrourae 1	
Leptotila verreauxi fulviventris	*Columbicola macrourae* 1	*Physconelloides ceratoceps* 3
	Columbicola gracilicapitis	

AF182703, AF182706, AF279704-AF279743). For wing lice (*Columbicola*), we sequenced 1,107 base pairs, including portions of the mitochondrial COI and 12S ribosomal genes, as well as the nuclear elongation factor 1-α gene (EF1α). We used the primers L6625 and H7005 (Hafner, et al., 1994) for COI, 12Sai and 12Sbi (Simon et al., 1994) for 12S, and EF1-For3 and EF1-Cho10 (Danforth and Ji, 1998) for EF1α (Genbank accession numbers AF190409, AF190411, AF190412, AF190416, AF190418, AF190420, AF190423, AF190424, AF190426, AF278608-AF278643). For body lice, we sequenced 737 base pairs, including portions of the mitochondrial COI gene and nuclear EF1α gene using the primers listed above (Genbank accession numbers AF278644-AF278679).

We sequenced several individual lice of each species for COI. The COI sequences revealed divergent monophyletic lineages within several

described morphological species of lice (Johnson et al., 2002). The divergence between lineages ranged from 3% to 18% uncorrected sequence divergence. However, within each of these lineages, COI sequences were identical or differed at only a few base positions (generally less than 1% sequence divergence). Thus, we sequenced only one representative individual from each of these lineages for EF1α and 12S. We do not give the divergent lineages unique names here, but designate them using arbitrary numbers within each morphological species (e.g., *Columbicola macrourae* 1). These numbered lineages were used as the terminals for cospeciation analyses.

Phylogeny Construction and Comparison

For all three sets of taxa (hosts, wing lice, and body lice), we constructed phylogenies using several different methods in the program PAUP* (Swofford, 1999). In all analyses, we combined gene regions for each taxon. We first reconstructed unordered parsimony trees. Next we used these trees to estimate the best fit maximum likelihood model using the general procedure of likelihood ratio tests as described by Huelsenbeck and Crandall (1997). In each case, the best fit maximum likelihood model incorporated six substitution categories (general time reversible), empirically estimated base frequencies, and rate heterogeneity under the gamma distribution (we partitioned the gamma distribution into eight rate categories). We also used this model with two substitution categories to estimate the transition:transversion ratio under maximum likelihood. We used this estimate (rounded to the nearest whole number) as a weight on transversions in parsimony searches. For both parsimony analyses, we constructed 100 bootstrap replicates (Felsenstein, 1985) to evaluate the relative support for branches in the trees.

To reconstruct trees under maximum likelihood, we used quartet puzzling (Strimmer and von Haeseler, 1996) as a shortcut to heuristic maximum likelihood searches. We used the model derived above in each case for the quartet puzzling replicates, employing a setting of 10,000 puzzling steps because of the relatively large number of taxa in each data set. We also used the reliability values as an indication of relative support for branches in the maximum likelihood analysis. As a third phylogeny reconstruction technique, we used neighbor joining with Kimura two-parameter (Kimura, 1980) distances.

For each type of analysis (unweighted parsimony, transversion weighted parsimony, maximum likelihood quartet puzzling, and neighbor joining) we compared host and parasite trees. Reconciliation analysis (Page, 1990, 1994a) as implemented in the computer program TREEMAP (Page, 1994b)

was used to determine the number and position of cospeciation events. We used a randomization of parasite trees to test whether there were significantly more cospeciation events than expected by chance (Page, 1990b, 1994b). These methods assume that both host and parasite phylogenies are known with certainty. However, all the trees had nodes with relatively low support (bootstrap or reliability scores). Thus, to take into account incongruence between phylogenies that might be attributable to weakly supported conflicting nodes (Huelsenbeck et al., 1997), we used the partition homogeneity test (Farris et al., 1994, 1995) and a taxon deletion method (Johnson, 1997; Johnson et al., 2001). Using this method, we assessed which associations resulted in significant incongruence between host and parasite trees. For example, some parasites are associated with multiple, sometimes unrelated, hosts. These associations are likely to cause significant incongruence between host and parasite phylogenies. We removed these incongruent host-parasite associations (sometimes entire taxa) and constructed a combined evidence tree for hosts and the relevant louse taxon. We then constrained this tree and added back in the removed taxa. We conducted parsimony searches with either the host or parasite data set under this constraint. Finally, we used these complete constrained trees in the TREEMAP analyses as indicated above.

To assess whether cospeciation events were correlated between wing and body lice, we tallied host nodes as having (1) no cospeciation events, (2) cospeciation with wing lice only, (3) cospeciation with body lice only, or (4) cospeciation with both wing and body lice. We used these values in a Fisher's exact test for independence. We conducted this test using all five comparisons of host and parasite phylogenies (unordered parsimony, transversion weighted parsimony, maximum likelihood, neighbor joining, and partition homogeneity test/taxon deletion method).

Results

Host Phylogeny

The phylogeny for pigeons and doves resulting from combined analysis of genes is completely resolved and well supported. The maximum likelihood quartet puzzling and unweighted parsimony trees were identical, and this tree was consistent with an analysis of a larger set of taxa (Johnson and Clayton, 2000). In the unweighted parsimony tree, 15 of 17 nodes were supported in greater than 50% of bootstrap replicates. In the quartet puzzling tree (fig. 11.2), all nodes had a reliability index greater than 50%. The transversion weighted parsimony and neighbor joining trees were similar

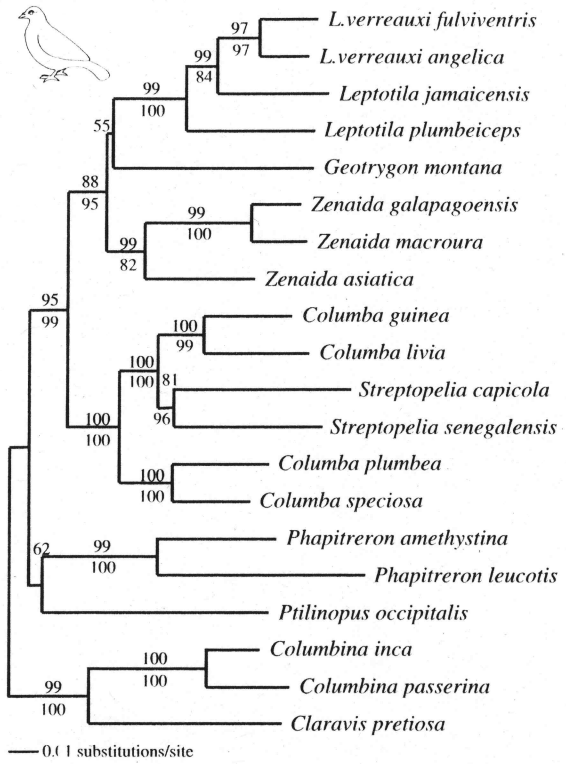


FIGURE 11.2. Phylogeny of Columbiformes derived from maximum likelihood quartet puzzling of COI, cyt *b*, and FIB7 sequences. Model parameters: empirical base frequencies with rate heterogeneity, gamma shape parameter = 0.224, eight rate categories, general time reversible model with transformation parameters 1.39 (A-C), 7.30 (A-G), 0.84 (A-T), 0.71 (C-G), 9.88 (C-T), 1.0 (G-T). Branch lengths are proportional to lengths estimated under the maximum likelihood model (scale indicated). Numbers below branches indicate bootstrap support from 100 replicates using unordered parsimony. Branches unlabeled below had bootstrap support less than 50%. Numbers above the branches indicate reliability indices from 10,000 puzzling replicates.

to the maximum likelihood tree, with most of the differences involving rearrangements of weakly supported nodes. With the exception of *Columba*, in all trees, Columbiform genera are monophyletic. Old World *Columba* are sister to *Streptopelia*, and New World *Columba* are sister to Old World *Columba* + *Streptopelia*. The minimum and maximum pairwise COI sequence divergences between species of Columbiformes were 3.3% and 15.4%, respectively.

Wing Louse Phylogeny

The single unweighted parsimony tree from combined gene regions for *Columbicola* was completely resolved. Of 12 nodes in the tree, 9 received bootstrap support in over 50% of replicates. The maximum likelihood quartet puzzling tree (fig. 11.3) was similar to this tree, with rearrangements involving weakly supported nodes, and this tree was generally well supported by the quartet puzzling reliability index. The transversion-weighted and neighbor-joining trees differed from these trees in the placement of weakly supported nodes.

Most morphologically described species of *Columbicola* were monophyletic; however, *Columbicola macrourae* was paraphyletic with respect

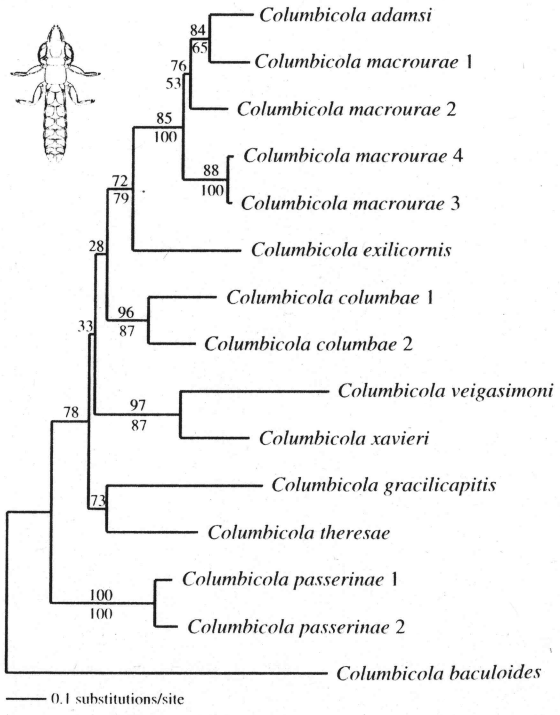


FIGURE 11.3. Phylogeny of wing lice *(Columbicola)* derived from maximum likelihood quartet puzzling of COI, 12S, and EF1α sequences. Model parameters: empirical base frequencies with rate heterogeneity, gamma shape parameter = 0.193, eight rate categories, general time reversible model with transformation parameters 0.53 (A-C), 6.46 (A-G), 1.86 (A-T), 1.39 (C-G), 10.92 (C-T), 1.0 (G-T). Conventions as in figure 11.2 (branch length scale indicated). One branch with 50% bootstrap support in the unordered parsimony analysis is not present in this tree.

to *Columbicola adamsi* in the unweighted parsimony, maximum likelihood, and neighbor joining trees (fig. 11.3). In the transversion weighted parsimony tree, *Columbicola adamsi* fell just outside *Columbicola macrourae*. The EF1α sequences for these two species were identical, so they are undoubtedly closely related. The minimum and maximum pairwise COI sequence divergences between lineages of *Columbicola* were 3.1% and 29.8%, respectively.

Body Louse Phylogeny

Unweighted parsimony analysis of body louse sequences produced a single completely resolved tree. However, several nodes of this tree were not well supported; only 6 of 15 nodes had over 50% support from bootstrap replicates. Furthermore, several relationships changed across the analyses. The maximum likelihood quartet puzzling analysis produced a tree (fig. 11.4) with 11 of 15 nodes receiving a reliability score greater than 50%. In a comparison of this tree with that from unordered parsimony, 8 of 16 nodes were identical between maximum likelihood and parsimony analyses.

Even though the body louse tree was not well supported overall, several relationships were consistent across analyses. The genus *Coloceras* was monophyletic in all analyses. Monophyly of *Coloceras* had strong support by the reliability index (98%), although not by bootstrapping (<50%). All four *Physconelloides* species groups (Price et al., 1999) represented in the maximum likelihood tree (fig. 11.4) were monophyletic. In all analyses, *P. ceratoceps* was paraphyletic, with *P. cubanus* falling within the three divergent *P. ceratoceps* lineages. *Physconelloides* was paraphyletic in all analyses, such that the other genera of body lice (*Auricotes, Campanulotes,* and *Coloceras*) were derived from within *Physconelloides*. The minimum and maximum pairwise COI sequence divergences between lineages of body lice were 3.6% and 19.3%, respectively.

Comparison of Host-Parasite Phylogenies

Comparing host and parasite phylogenies often reveals instances of cospeciation (Hafner and Nadler, 1988; Hafner et al., 1994; Moran and Baumann, 1994; Paterson and Gray, 1997; Page et al., 1998). Host-parasite cospeciation results from concurrent isolation of host and parasite populations, resulting in congruent phylogenies. Incongruence between host and parasite phylogenies can arise from several processes that are difficult to distinguish, making the interpretation of incongruence relatively difficult.

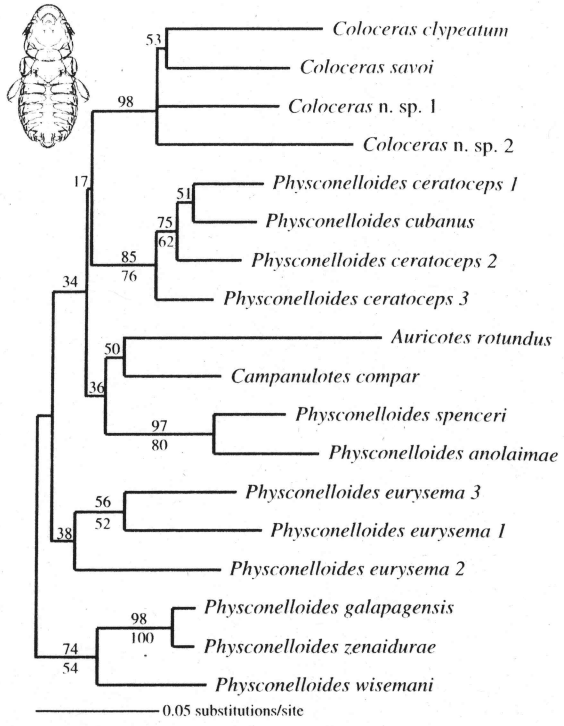


FIGURE 11.4. Phylogeny of body lice *(Auricotes, Campanulotes, Coloceras, Physconelloides)* derived from maximum likelihood quartet puzzling of COI and EF1α sequences. Model parameters: empirical base frequencies with rate heterogeneity, gamma shape parameter = 0.104, eight rate categories, general time reversible model with transformation parameters 0.02 (A-C), 13.05 (A-G), 3.87 (A-T), 0.50 (C-G), 5.48 (C-T), 1.0 (G-T). Conventions as in figure 11.2 (branch length scale indicated; note difference from fig. 11.2). One branch with 50% bootstrap support in the unordered parsimony analysis is not present in this tree.

Events other than cospeciation can often be difficult to infer with certainty, and often several possible reconstructions exist for any given host and parasite trees. For this reason, in our comparisons of the phylogenies of Columbiformes and their lice, we have chosen to focus on cospeciation events. Reconciliation analysis (Page, 1990a, 1994a) is a straightforward method for recovering cospeciation events. Although it does not allow for host switching, reconciliation analysis is sufficient for the goal of this study, which was to compare the coevolutionary histories of ecological replicates.

We compared trees of hosts and wing lice resulting from each of our five types of phylogenetic analysis (see Methods). In each comparison, we

recovered eight cospeciation events (e.g., fig. 11.5A). In each case, there were more cospeciation events between Columbiformes and *Columbicola* than expected by chance ($p < 0.01$ for all five analyses). The two most basal nodes in the host phylogeny showed cospeciation in all five analyses. The *Columbicola* node cospeciating with the basal host node was not the most basal *Columbicola* node, but was higher up in the tree. The most basal *Columbicola* node never showed cospeciation. Three host speciation events always showed cospeciation: (1) *Zenaida macroura—Z. galapagoensis,* (2) *Columba livia—C. guinea,* and (3) *Claravis—Columbina.* Although the lice in these three cases of cospeciation are conspecific on morphological grounds (Clayton and Price, 1999), their DNA sequences are highly divergent (fig. 11.3).

For body lice, eight cospeciation events (fig. 11.5B) were recovered by the four methods of analysis that did not exclude weakly supported nodes. In contrast, 10 cospeciation events were recovered when weakly supported nodes were taken into account using the partition homogeneity test/taxon deletion method (see Methods). Eight cospeciation events were more than expected by chance ($p = .05$), or nearly so ($p = .07$), depending on the type of phylogenetic analysis. Ten cospeciation events were considerably more than expected by chance ($p = .003$). As for the wing lice, body lice showed cospeciation with the two most basal nodes in the host tree, regardless of analytical method. Other cospeciation events consistent across analyses included one event involving *Columbina,* one involving *Columba,* and two events involving *Zenaida.* In the case of the four straight tree comparisons, the most basal node in the parasite tree did not show cospeciation. However, the fifth method (partition homogeneity test/taxon deletion) recovered basal cospeciation of body lice.

Testing Independence of Cospeciation Events

We tested for the independence of wing and body louse cospeciation events by evaluating the host nodes that showed no cospeciation, cospeciation in one taxon only, and cospeciation in both wing and body lice (see table 11.2 for an example using the maximum likelihood trees). For all five analyses, the two-tailed *p*-value (Fisher's exact test) was 1.0, indicating that speciation events in wing and body lice are independent. Out of 19 host nodes, only 3 or 4, depending on the analysis, exhibited cospeciation with both wing and body lice.

If cospeciation events in the two parasite groups were correlated, we would expect the parasite phylogenies themselves to be somewhat congruent. However, the parasite phylogenies are largely incongruent. For

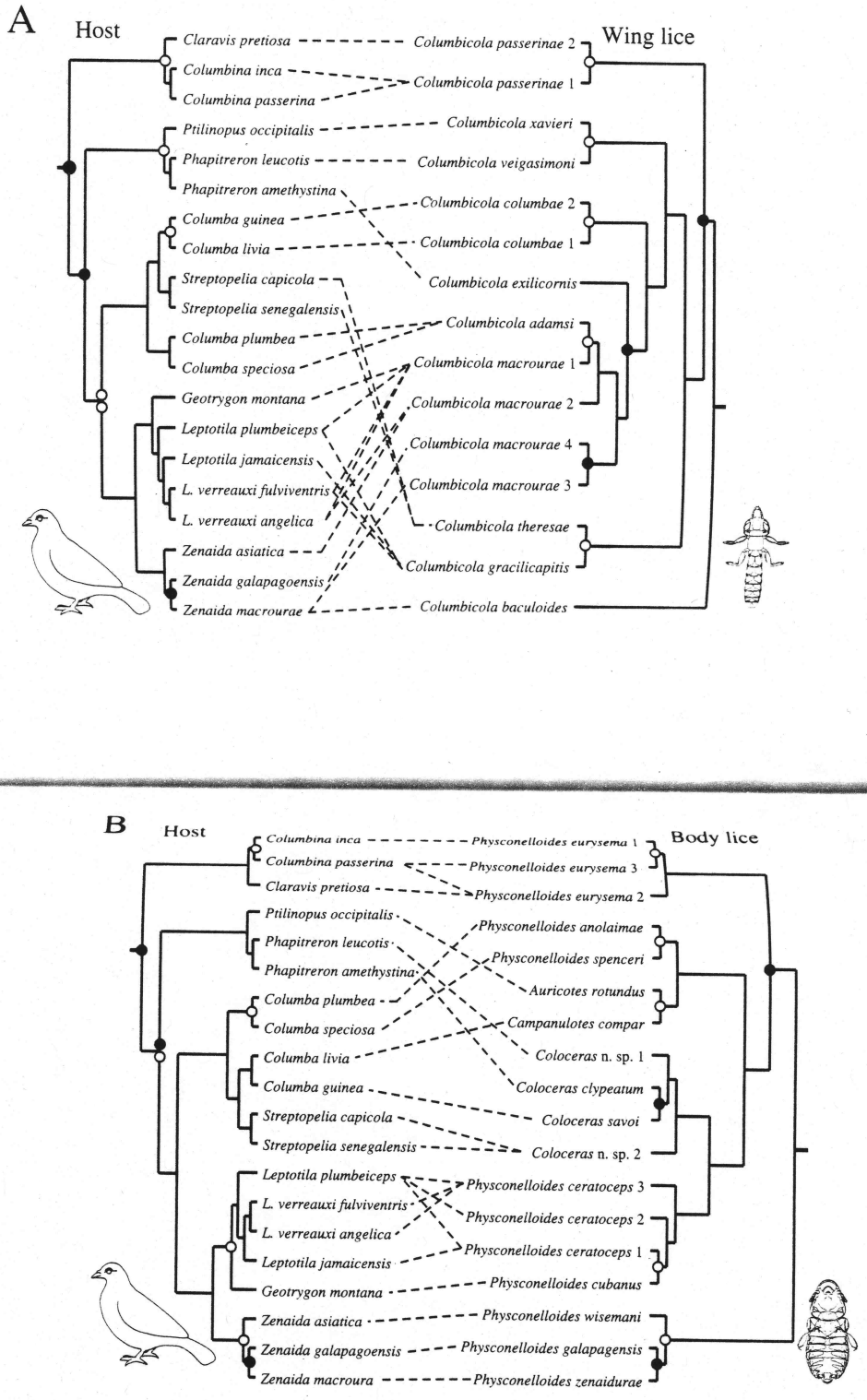


FIGURE 11.5. Comparison of quartet puzzling maximum likelihood trees for Columbiformes and *(A)* wing lice and *(B)* body lice. Lines connecting taxa indicate host-parasite associations. Circles represent nodes inferred by reconciliation analysis to have cospeciated. Closed circles are nodes that are cospeciation events shared by wing and body lice. Open circles are nodes that are not shared cospeciation events.

TABLE 11.2 Comparison of nodes with and without cospeciation in wing vs. body lice

		Body Lice	
		Cospeciation	No Cospeciation
Wing lice	Cospeciation	3	4
	No cospeciation	4	8

Fisher's exact test $p = 1.0$. Quartet puzzling maximum likelihood trees used in the analysis.

example, the wing lice on the host sister species *Columba plumbea* and *C. speciosa* are the same species, having failed to speciate, while the body lice on these hosts are sister taxa. Another example is that the wing lice on the host sister species *Columba livia* and *C. guinea* are also sister species, whereas the body lice on these same hosts are in different genera. In summary, concordance between wing and body louse phylogenies is minimal.

Discussion

Some portions of the host-parasite trees we reconstructed showed evidence of cospeciation, whereas other portions were incongruent. Cospeciation events in wing and body lice were not significantly correlated, suggesting that factors promoting cospeciation in wing lice may be independent of those promoting cospeciation in body lice. A number of issues relevant to comparisons of host and parasite phylogenies are evidenced by this study. We explore each of these issues below.

Estimating the Frequency of Events in Host-Parasite Histories

Reconciliation analysis (Page, 1990a, 1994a) identifies three types of events when comparing host and parasite phylogenies: cospeciation, parasite duplication, and sorting events (such as parasite extinction). A more refined analysis (TREEMAP: Page, 1994b) allows for the possibility of host switching. In our analysis, we observed a fifth phenomenon not explicitly incorporated into existing tree comparison algorithms: failure to speciate. We uncovered three instances of wing lice failing to cospeciate with speciating hosts. We also found two instances of failure to speciate in body lice. Analyses comparing host and parasite trees make assumptions about the relative frequency of historical events when arriving at an optimal reconstruction. However, it is largely unknown how common each of the five types of events listed above are in nature.

A conservative way to evaluate the frequency of cophylogenetic events is to examine terminal sister taxa. Terminal taxa comparisons circumvent many of the difficulties of phylogenetic inference for deeper nodes because

these comparisons are independent of one another, as well as of other nodes in the tree. We can evaluate the relative frequency of cospeciation, parasite duplication, sorting, and failure to speciate by examining relationships of the lice on terminal host sister taxa. The frequency of host switching cannot be evaluated using this approach, since by definition, host switching involves nonsister species of hosts. We examined seven pairs of terminal host sister taxa (*Columbina, Phapitreron,* New World *Columba, Streptopelia,* Old World *Columba, Leptotila verreauxi,* and *Zenaida*) and recorded whether their associated lice showed (1) cospeciation, (2) failure to speciate, or (3) other incongruence events with multiple possible explanations. We also evaluated the relative frequency of parasite duplication (speciation in the parasite not accompanied by host speciation) by examining each host species and determining whether a speciation event had occurred between its associated parasites (table 11.3). We were not able to evaluate the relative frequency of sorting events for the two parasite groups because, in our study, we intentionally included species of hosts from which we had samples of both wing and body lice. Nearly all species of Columbiformes that have been thoroughly sampled are known to have both wing and body lice, so recent sorting events appear to be rare. However, no wing lice have been found on one well-sampled species, the New Zealand Pigeon *(Hemiphaga novaeseelandiae)* (Paterson et al., 1999; R. Palma, pers. comm.). To our knowledge, this is the only evidence suggesting a possible extinction of feather lice on an extant Columbiform host. Another interesting case is the extinct Passenger Pigeon *(Ectopistes migratorius),* from which body lice have never been recovered, despite concerted efforts to find them on museum skins from which many wing lice have been collected (Price et al., 2000).

In the case of wing lice from terminal host sister taxa, we observed two cospeciation events, three failure to speciate events, and two other incongruence events (involving deeper combinations of duplications, sorting events, and/or host switches: table 11.3). We found no evidence of wing

TABLE 11.3 Numbers of cophylogenetic events for host sister taxa comparisons

Event	Wing Lice	Body Lice
Cospeciation	2	3
Failure to speciate	3	2
Other incongruence event(s)	2	2
Duplications	0	0

Note: Duplications based on examination of 20 terminal host taxa. All other events based on seven congeneric sister taxa comparisons.

louse duplication on any of the 20 extant species of hosts. For the seven comparisons of body lice on terminal host sister taxa (table 11.3), we found three cases of cospeciation, two failure to speciate events, and two other incongruence events. Again, we found no evidence of body louse duplication on any of the 20 extant host species.

Our evaluation of events in closely related taxa suggests that failure to speciate may be a seriously overlooked event in reconciling host and parasite phylogenies. Conversely, it appears that the importance of parasite duplication may be overemphasized when seeking explanations to reconcile host and parasite phylogenies. It may be that by inferring failure to speciate rather than parasite duplication, host and parasite phylogenies could be more easily reconciled (in terms of the number of events needed to explain the differences). For example, consider the hypothetical biogeographic scenario depicted in figure 11.6. The once contiguous host species A is fragmented by a geographic barrier, and the host speciates into A and B. However, there could still be sufficient gene flow between parasite populations to prevent speciation in the parasite X (a "failure to speciate" event). This scenario is not unreasonable; for example, Dybdahl and Lively (1996) found much higher levels of gene flow between populations in a trematode parasite than in its snail host. (In lice, failure to speciate might occur by phoresis of lice on hippoboscid flies between diverging host populations.) If host species A then colonizes a new isolated area by dispersal, one could imagine a new speciation event in the host (producing host species C). Coincident with host speciation is a speciation event in the parasite (producing parasite species Y) because of a complete lack of further gene flow between the more completely isolated parasite populations. This scenario would produce the host-parasite phylogenies shown in figure 11.6. Using TreeMap for host-parasite history reconstruction, and invoking cospeciation, duplication, sorting, and host switching, we recovered four events needed to explain the pattern: one duplication and three sorting events. However, allowing the parasite to fail to speciate, only two events are needed: a failure to speciate event and a cospeciation event. We suggest that future work on methods of host-parasite phylogeny reconciliation explicitly take into account failure of the parasite to speciate as a possible event in the history of the host-parasite association.

Host Specificity and the Significance of Cospeciation

For all comparisons of host and parasite trees we found the same number or more cospeciation events in body lice than in wing lice. However, in most analyses body lice showed only a marginally significant amount of

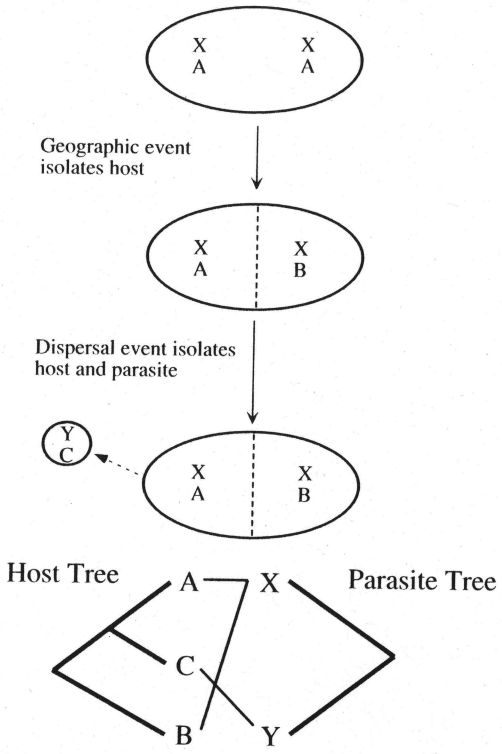


FIGURE 11.6. Hypothetical scenario for speciation in host and parasites and resulting phylogenies. Contiguous host species A fragments by a geographic barrier into host species A and host species B. Parasite species X is not affected by this barrier and fails to speciate. Subsequently, host species A colonizes an isolated area and this results in speciation in both the host and parasite, producing host C and parasite Y.

cospeciation, while the p-value for wing lice was always low ($< .01$). How can these differences be explained?

The first possibility is that we have the wrong body louse phylogeny. Many of the nodes in the body louse tree are poorly supported compared with the trees for the hosts and wing lice. This poor support is most likely a result of the fact that we have the least amount of total sequence for body lice. A further indication that an incorrect louse phylogeny may be a contributing factor is the marginal significance of cospeciation when the body louse phylogeny is taken to be correct, compared with the strongly significant cospeciation observed when we used the partition homogeneity test/taxon deletion method. This method explicitly takes into account differences between host and parasite trees owing to weak support (Johnson et al., 2001).

However, the fact that the same number of cospeciation events (eight) between wing and body lice occurs in most of the comparisons suggests there might be an additional explanation for the differences in *p*-values. The basic technique for evaluating whether more cospeciation is observed than expected by chance is to randomize the parasite phylogeny and count the number of resulting "cospeciation" events (Page, 1990b, 1994b). This procedure produces a null distribution with which the observed number of cospeciation events can be compared. An example of these distributions is shown in figure 11.7A for the maximum likelihood trees for both wing and body lice. The distribution for body lice is shifted to the right, compared with the distribution for wing lice. One possible explanation for this shift in the null distribution is that body lice are more host specific than wing lice. High host specificity may tend to make recovering a large number of cospeciation events more likely by chance. To examine the impact of host specificity on the null distribution, we arbitrarily pruned host associations from the body louse and host trees, making each body louse species perfectly host specific. When this is done, the distribution shifts even further to the right; the number of randomizations with a high number of cospeciation events increases, while the number of randomizations with a low number of cospeciation events decreases (fig. 11.7B). This effect, when combined with the fact that there are more body louse species (18) than wing louse species (15) on the same hosts, may explain why wing lice showed highly significant cospeciation, while body lice generally showed marginal *p*-values. While the shift in the randomized distribution due to increased host specificity is not dramatic, it has the potential to alter the significance level assigned to the amount of cospeciation recovered. These observations indicate that caution should be used when comparing the results of randomization tests for hosts and parasites across parasite groups that differ in host specificity.

Relative Ages of Host-Parasite Associations

A striking difference between the wing and body louse sequence data sets is that wing lice were much more divergent than body lice (note branch lengths and difference in scales between figs. 11.3 and 11.4). Pairwise uncorrected sequence divergences between wing louse species generally range between 18% and 30% for COI, and between 0% and 11% for EF1α. In contrast, pairwise uncorrected sequence divergences between body louse species generally range between 8% and 20% for COI, and between 0% and 3% for EF1α. One possible explanation for this difference is that wing lice are evolving faster at the molecular level than body lice. However, examination of a few correlated recent cospeciation events indicates that, if anything, body lice are evolving faster than wing lice.

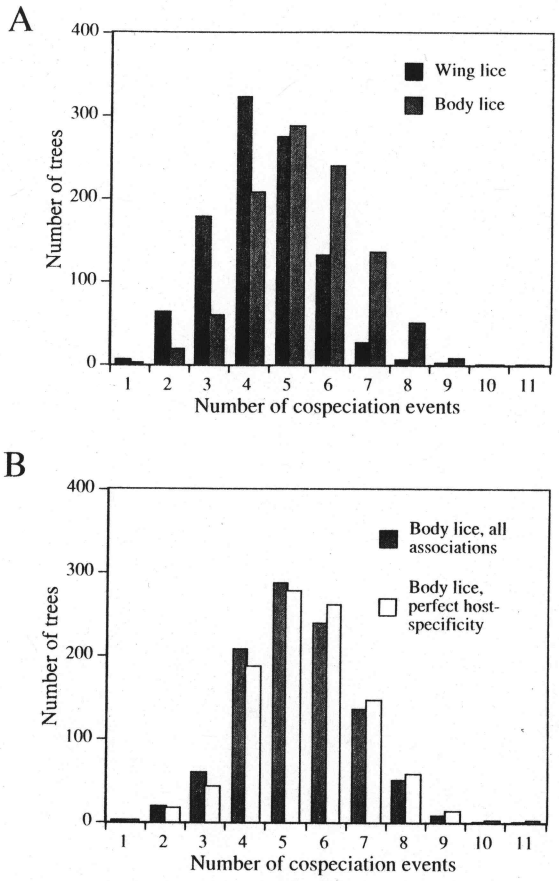


FIGURE 11.7. Comparison of distributions of number of cospeciation events for 1,000 random louse trees: *A*, wing lice and body lice; *B*, body lice with all host associations maintained and body lice with host associations removed such that each species of louse is found on only one host.

A second possibility is that Columbiform wing lice are an older radiation than Columbiform body lice. In the partition homogeneity test/taxon deletion analysis, which has the highest probability of inferring cospeciation events, the first speciation event in wing lice is not a cospeciation event, while it is a cospeciation event in body lice. If the oldest node in the Columbiform phylogeny is a cospeciation event, but the oldest node in the *Columbicola* phylogeny is not one, then *Columbicola* must be older than modern Columbiform hosts. If modern *Columbicola* lineages did evolve before modern Columbiform lineages, this suggests there may be lineages of hosts previously parasitized by these old *Columbicola* lineages, but which

are now extinct. These old *Columbicola* lineages may have survived by colonizing more recently evolving host species, and this possibility is consistent with the broad host distribution of many *Columbicola* species.

Independence of Cospeciation Events

We found that cospeciation events between wing and body lice were largely independent. Two of the three cospeciation events common to both wing and body lice were the two most basal nodes of the host tree. While this may reflect actual history, it also seems probable that this may be an artifact of the way reconciliation methods work. When host and parasite trees are not completely congruent, reconciliation methods tend to map shallow parasite speciation events onto deep host speciation events. This has a tendency to "push" cospeciation events back in the host tree, which may explain why basal nodes in the host tree often showed cospeciation in our analyses. If this is the case, one should be cautious when using deep cospeciation events to compare rates of evolution between hosts and parasites.

The third cospeciation event common to both wing and body lice involved the node between the Mourning Dove *(Zenaida macroura)* and Galapagos Dove *(Z. galapagoensis)*. Since the Galapagos Dove is the only species of dove on the Galapagos Islands, it probably brought its lice with it upon colonization. This biogeographic event, which was common to both wing and body lice, would have caused speciation in both of them. When biogeographic isolating events are responsible for speciation in the host, and this isolation is extreme as in the colonization of an island, we would expect speciation events in replicate parasite groups to be correlated. However, when hosts and parasites are more broadly distributed, isolating events for one parasite group may not affect the other, even when the parasites share similar ecologies.

Understanding reasons for the similarities (few as they are) between the two parasite trees is easier than understanding their differences. One possibility is that the differences arise from differences in chance events (e.g., chance parasite extinction). In such a case, we would expect to see little pattern to the differences. On the other hand, there may be predictable patterns underlying the differences between parasite phylogenies. We suspect that wing lice are less likely to speciate than body lice in response to host speciation, which gives rise to the differences in phylogenies. Although body lice showed only one less failure to speciate event than wing lice (table 11.3), population level genetic data indicated significant differentiation in one of the cases (*Physconelloides ceratoceps* 3 on *Leptotila verreauxi fulviventris* and *L. v. angelica:* Johnson et al., 2002). Perhaps *P. ceratoceps* 3

is cospeciating with its hosts, but is lagging behind in the process. Unlike body lice, no genetic differentiation can be detected in populations of *Columbicola* on multiple species of host, suggesting that *Columbicola* is capable of dispersing to multiple hosts (Johnson et al., 2002). Wing lice can survive for a longer period of time off the host than body lice (unpub. data), which is consistent with a higher probability of dispersal by wing lice. Ecological studies comparing dispersal of wing and body lice among host species are needed to confirm this differential dispersal hypothesis. The lower dispersal ability of body lice could cause them to speciate in response to host speciation more often than wing lice. Uncovering this possible link between ecology and macroevolutionary pattern is an exciting future prospect, which we discuss in chapter 13.

Acknowledgments

We are extremely grateful to the following individuals and institutions who provided critical assistance, without which the fieldwork would not have been possible: D. Drown, L. Heaney, N. Ingle, R. Moyle, A. Navarro, A. T. Peterson, M. Robbins, J. Weckstein, and Texas Parks and Wildlife. R. J. Adams and R. D. Price prepared slide mounts and provided identifications for the voucher specimens. We are grateful to J. Burtt and J. Ichida for the SEMs of lice, V. Smith for line drawings of lice, and S. Al-Tamimi for line drawings of Columbiformes. We thank R. J. Adams, D. Drown, and P. Ewald, R. Page, and A. Paterson for valuable discussion or comments on the manuscript. Several host tissue samples were provided by the Kansas Museum of Natural History, the Field Museum of Natural History, the Louisiana State University Museum of Natural Science, and the Tracy Aviary. We thank the DNA Sequencing Facility at the University of Utah, supported in part by NCI grant #5p30CA42014. This work was supported by NSF-CAREER award DEB-9703003 to D.H.C.

References

Booth, T. D., 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,* ser. B, 253:125–29.

Brooks, D. R., and D. A. McLennan. 1991. *Phylogeny, ecology, and behavior.* Chicago.: University of Chicago Press.

Clayton, D. H. 1991. Coevolution of avian grooming and ectoparasite avoidance. In *Bird-parasite interactions: Ecology, evolution, and behaviour,* edited by J. E. Love and M. Zuk. Oxford: Oxford University Press.

Clayton, D. H., P. L. M. Lee, D. M. Tompkins, and E. D. Brodie III. 1999. Reciprocal natural selection on host-parasite phenotypes. *American Naturalist* 154:261–70.

Clayton, D. H., and R. D. Price. 1999. Taxonomy of New World *Columbicola* (Phthiraptera: Philopteridae) from the Columbiformes (Aves), with descriptions of five new species. *Annals of the Entomological Society of America* 92:675–85.

Clayton, D. H., and D. M. Tompkins. 1994. Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London*, ser. B 256:211–17.

———. 1995. Comparative effects of lice and mites on the reproductive success of rock doves *(Columba livia)*. *Parasitology* 110:195–206.

Clayton, D. H., and B. A. Walther. 1997. Collection and quantification of arthropod parasites of birds. In *Host-parasite evolution: General principles and avian models*, edited by D. H. Clayton and J. Moore, 419–40. Oxford: Oxford University Press.

Couch, A. B. J. 1962. Phoretic mallophaga from hiposboscids of mourning doves *Zenaidura macroura*. *Journal of Parasitology* 48:497.

Cruickshank, R. H., K. P. Johnson, V. S. Smith, R. J. Adams, D. H. Clayton, and R. D. M. Page. 2001. Phylogenetic analysis of partial sequences of elongation factor 1 alpha identifies major groups of lice (Insecta: Phthiraptera). *Molecular Phylogenetics and Evolution* 19:202–15.

Danforth, B. N., and S. Ji. 1998. Elongation factor-1α occurs as two copies in bees: Implications for phylogenetic analysis of EF-1α sequences in insects. *Molecular Biology and Evolution* 15:225–35.

Dybdahl, M. F., and C. M. Lively. 1996. The geography of coevolution: Comparative population structures for a snail and its trematode parasite. *Evolution* 50:2264–75.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10:315–19.

———. 1995. Constructing a test for incongruence. *Systematic Biology* 44:570–72.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–91.

Hafner, M. S., and S. A. Nadler. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* 332:258–59.

Hafner, M. S., P. D. Sudman, F. X. Villablanca, T. A. Spradling, J. W. Demastes, and S. A. Nadler. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* 265:1087–90.

Hoberg, E. P., D. R. Brooks, and D. Seigel-Causey. 1997. Host-parasite cospeciation: History, principles, and prospects. In *Host-parasite evolution: General principles and avian models*, edited by D. H. Clayton and J. Moore, 212–35. Oxford: Oxford University Press.

Hopkins, G. H. E., and T. Clay. 1952. A checklist of the genera and species of Mallophaga. London: British Museum of Natural History.

Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* 28:437–66.

Huelsenbeck, J. P., B. Rannala, and Z. Yang. 1997. Statistical tests of host-parasite cospeciation. *Evolution* 51:410–19.

Johnson, K. P. 1997. The evolution of behavior in the dabbling ducks (Anatini): A phylogenetic approach. Ph.D. diss., University of Minnesota.

Johnson, K. P., and D. H. Clayton. 2000. Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (Aves: Columbiformes). *Molecular Phylogenetics and Evolution* 14:141–51.

Johnson, K. P., D. M. Drown, and D. H. Clayton. 2001. A data based parsimony method of cophylogenetic analysis. *Zoologica Scripta* 30:79–87.

Johnson, K. P., B. L. Williams, D. M. Drown, R. J. Adams, and D. H. Clayton. 2002. The population genetics of host specificity: Genetic differentiation in dove lice (Insecta: Phthiraptera). *Molecular Ecology* 11:25–38.

Kimura, M. 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–20.

Marshall, A. G. 1981. *The ecology of ectoparasitic insects*. London: Academic Press.

Moran, N., and P. Baumann. 1994. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. *Trends in Ecology and Evolution* 9:15–20.

Nelson, B. C., and M. D. Murray. 1971. The distribution of Mallophaga on the domestic pigeon *(Columba livia)*. *International Journal for Parasitology* 1:21–29.

Page, R. D. M. 1990a. Component analysis: A valiant failure? *Cladistics* 6:119–36.

———. 1990b. Temporal congruence and cladistic analysis of biogeography and cospeciation. *Systematic Zoology* 39:205–26.

———. 1994a. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. *Systematic Biology* 43:58–77.

———. 1994b. Parallel phylogenies: Reconstructing the history of host-parasite assemblages. *Cladistics* 10:155–73.

Page, R. D. M., and M. A. Charleston. 1998. Trees within trees: Phylogeny and historical associations. *Trends in Ecology and Evolution* 13:356–59.

Page, R. D. M., P. L. M. Lee, S. A. Becher, R. Griffiths, and D. H. Clayton. 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Molecular Phylogenetics and Evolution* 9:276–93.

Paterson, A. M., and R. D. Gray. 1997. Host-parasite cospeciation, host switching, and missing the boat. In *Host-parasite evolution: General principles and avian models*, edited by D. H. Clayton and J. Moore, 236–50. Oxford: Oxford University Press.

Paterson, A. M., R. L. Palma, and R. D. Gray. 1999. How frequently do avian lice miss the boat? *Systematic Biology* 48:214–23.

Price, R. D., R. J. Adams, and D. H. Clayton. 2000. Pigeon lice down under: Taxonomy of the Australian *Campanulotes* (Phthiraptera: Philopteridae), with a description of *C. durdeni* n. sp. *Journal of Parasitology* 86:948–50.

Price, R. D., D. H. Clayton, and R. A. Hellenthal. 1999. Taxonomic review of *Physconelloides* (Phthiraptera: Philopteridae) from the Columbiformes (Aves), including descriptions of three new species. *Journal of Medical Entomology* 36:195–206.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and

a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87:651–704.

Smith, V. S. 2000. Basal ischnoceran louse phylogeny (Phthiraptera: Ischnocera: Gonioididae and Heptapsogasteridae). *Systematic Entomology* 25:73–94.

Strimmer, K., and A. von Haeseler. 1996. Quartet puzzling: A quartet maximum-likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution* 13:964–69.

Swofford, D. L. 1999. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods)*. Sunderland, Mass.: Sinauer Associates.