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When Do Parasites Fail to Speciate in Response to Host Speciation?

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Abstract.— Cospeciation generally increases the similarity between host and parasite phylogenies. Incongruence between host and parasite phylogenies has previously been explained in terms of host switching, sorting, and duplication events. Here, we describe an additional process, failure of the parasite to speciate in response to host speciation, that may be important in some host–parasite systems. Failure to speciate is likely to occur when gene flow among parasite populations is much higher than that of their hosts. We reconstructed trees from mitochondrial and nuclear DNA sequences for pigeons and doves (Aves: Columbiformes) and their feather lice in the genus *Columbicola* (Insecta: Phthiraptera). Although comparisons of the trees from each group revealed a significant amount of cospeciation, there was also a significant degree of incongruence. Cophylogenetic analyses generally indicated that host switching may be an important process in the history of this host–parasite association. Using terminal sister taxon comparisons, we also identified three apparent cases where the host has speciated but the associated parasite has not. In two of these cases of failure to speciate, these comparisons involve allopatric sister taxa of hosts whose lice also occur on hosts sympatric with both of the allopatric sisters. These additional hosts for generalist lice may promote gene flow with lice on the allopatric sister species. Relative rate comparisons for the mitochondrial cytochrome oxidase I gene indicate that molecular substitution occurs about 11 times faster in lice than in their avian hosts. [Coevolution; Columbiformes; cospeciation; lice; molecular phylogeny; Phthiraptera.]

Phylogenetic studies of interacting organisms often reveal congruence between the phylogenies of the interacting taxa (Hafner and Nadler, 1990; Moran and Baumann, 1994). This congruence is often ascribed to the phenomenon of cospeciation, i.e., concurrent speciation of both taxa involved in the interaction. In most cases, however, congruence between phylogenies of interacting taxa is not perfect. Such incongruence between phylogenies can result from several evolutionary processes (Fig. 1).

In host–parasite systems, three processes have been proposed to explain incongruence between host and parasite phylogenies (Page, 1994; Paterson and Gray, 1997; Page and Charleston, 1998). In host switching (Fig. 1f–i), one parasite switches either partially or completely to a new host. Parasite duplication (i.e., speciation of the parasite in the absence of host speciation) can also create incongruence between host and parasite phylogenies (Fig. 1c). A third process that promotes incongruence is the loss of a parasite lineage from a host lineage, i.e., a sorting event. Sorting events can involve extinction (Fig. 1d) or "missing the boat" (Fig. 1e, absence of parasites on a host population involved in founder event speciation). Here, we describe a fourth process that can, in combination with subsequent speciation or other events, lead to incongruence between host and parasite trees: failure of the parasite to speciate in response to host speciation (Clay, 1949; Paterson and Banks, 2001; Johnson and Clayton, 2002) (Fig. 1b). We provide evidence that failure to speciate can be common and suggest that this process should be considered when reconciling host and parasite phylogenies.

Parasitic lice (Insecta: Phthiraptera) are a model system for comparisons of host and parasite phylogenies. Lice spend their entire lifecycle on the host and exhibit a remarkable degree of host specificity. Given these properties, cospeciation should be the rule. However, in louse systems studied to date, cospeciation is never perfect (Barker, 1991; Hafner et al., 1994; Page et al., 1998; Paterson et al., 2000; Johnson et al., 2001d). An understanding of the evolutionary history of host–parasite associations in these cases requires an understanding of the evolutionary processes that lead to incongruence. One of the model systems for studies of louse ecology and coevolution is the louse genus *Columbicola* (Nelson and Murray, 1971; Eichler et al., 1972; Clayton, 1991; Clayton et al., 1999). By integrating studies of the biology of lice in this genus within a cophylogenetic framework, a more detailed understanding of the processes underlying macroevolutionary patterns is possible.

Species of *Columbicola* are parasites of pigeons and doves (Aves: Columbiformes). These lice are long and slender and can insert themselves between the barbs of the wing feathers to avoid preening by the host (Clayton, 1991). Most transmission is vertical, between parent and offspring, but individuals of *Columbicola* are also known to move phoretically by attaching to hippoboscid flies (Couch, 1962; Keirans, 1975). Although some species in this genus are completely host specific, others are found on multiple species of hosts (Hopkins and Clay, 1952; Clayton and Price, 1999; Johnson et al., 2002b). Johnson et al. (2002b) found genetic variation within *Columbicola* populations on different host species, but in many cases this variation was not structured according to host, providing evidence for ongoing gene flow.

We explored the coevolutionary history of *Columbicola* with Columbiformes by comparing the phylogenies of these two groups inferred from nuclear and mitochondrial gene sequences. We first evaluated the degree of congruence between host and parasite phylogenies, inferring from this the degree of cospeciation. We also reconstructed other cophylogenetic events using currently available methods. Using branches of the trees consistent with cospeciation, we estimated the relative rate

of molecular evolution between hosts and parasites. We then examined pairs of terminal sister host taxa and evaluated the status of their parasites to infer the relative frequency of cophylogenetic events. Specifically, we assessed how many times terminal sister pairs of host taxa harbor a single parasite species, providing evidence for failure to speciate.

METHODS

Samples and DNA Sequencing

We obtained samples of muscle tissue from various species of Columbiformes. From these same species, we removed lice by postmortem ruffling (Clayton and Drown, 2001). For our study, we included 27 host species and the associated 15 species of *Columbicola* (Table 1). Multiple divergent haplotypes (p distance >18%) for mitochondrial genes are observed within some species of *Columbicola*, and often these haplotypes are host specific (Johnson et al., 2002b). Thus, we used representatives of each of these haplotype groups in the study, resulting in 21 terminal taxa for *Columbicola*. We consider six of these taxa to be cryptic species but are currently examining specimens for morphological variation (see also Johnson et al., 2002b). For DNA extraction of host tissue, we followed procedures outlined by Johnson and Clayton (2000). For lice, we carefully removed the head from the body with a jeweler's forceps. We placed both parts in the DNA extraction buffer and proceeded with DNA extraction using a Qiagen Tissue Kit and the manufacturer's

TABLE 1. Samples of Columbiformes and *Columbicola* with host associations.

Host species	Parasite species
Columbina inca	*Columbicola passerinae* 1
Columbina passerina	*Columbicola passerinae* 1
Claravis pretiosa	*Columbicola passerinae* 2
Metriopelia ceciliae	*Columbicola gymnopeliae*
Phapitreron amethystina	*Columbicola exilicornis*
Phapitreron leucotis	*Columbicola veigasimoni*
Ptilinopus occipitalis	*Columbicola xavieri*
Patagioenas speciosa	*Columbicola adamsi*
Patagioenas plumbea	*Columbicola adamsi*
Patagioenas fasciata	*Columbicola extinctus*
Patagioenas subvinacea	*Columbicola macrourae* 5
Streptopelia decaocto	*Columbicola bacillus*
Streptopelia senegalensis	*Columbicola theresae*
Streptopelia capicola	*Columbicola theresae*
Streptopelia vinacea	*Columbicola theresae*
Oena capensis	*Columbicola theresae*
Turtur brehmeri	*Columbicola* n. sp.
Columba livia	*Columbicola columbae* 1
Columba guinea	*Columbicola columbae* 2
Zenaida asiatica	*Columbicola macrourae* 2
Zenaida macroura	*Columbicola macrourae* 3
	Columbicola baculoides
Zenaida galapagoensis	*Columbicola macrourae* 4
Geotrygon montana	*Columbicola macrourae* 1
Leptotila plumbeiceps	*Columbicola macrourae* 1
	Columbicola gracilicapitis
Leptotila jamaicensis	*Columbicola gracilicapitis*
Leptotila verreauxi angelica	*Columbicola macrourae* 1
Leptotila verreauxi fulviventris	*Columbicola macrourae* 1
	Columbicola gracilicapitis
Leptotila rufaxilla	*Columbicola timmermanni*

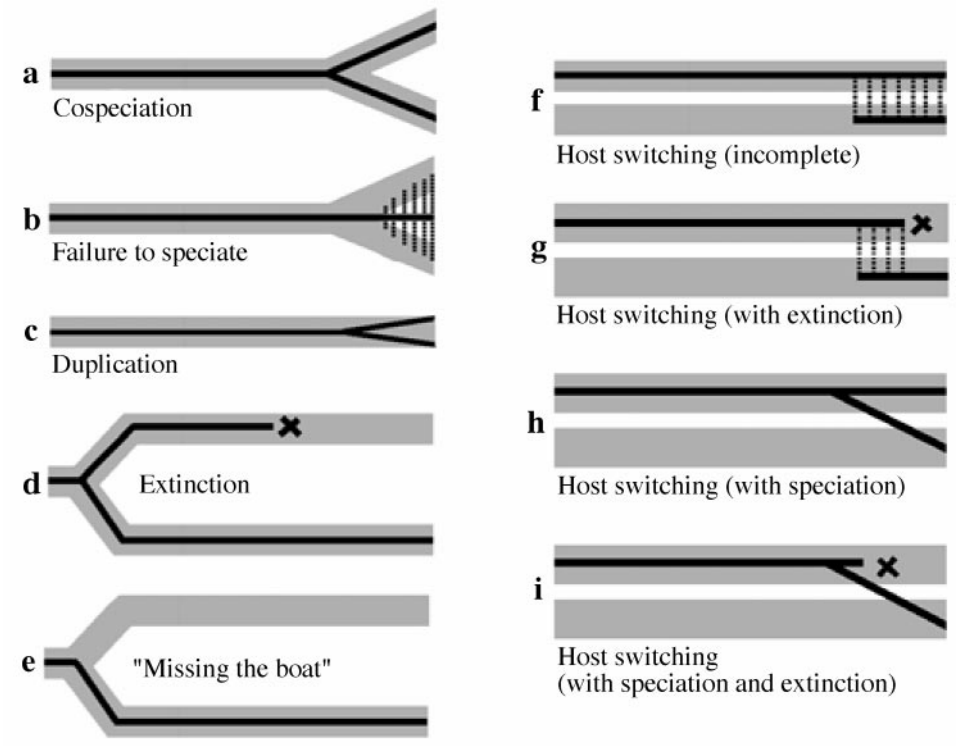

FIGURE 1. Cophylogenetic processes in host–parasite coevolutionary histories. Host lineages are shaded; parasite lineages are black lines. Dashed lines indicate gene flow of parasite populations between different host species.

protocols. However, we incubated the head and body of the louse in the digestion buffer for 56 hours. Upon digestion, we removed the head and the body of the louse from the buffer and reassembled them by mounting them together on a microslide. These slide-mounted specimens retain all the necessary features needed for identification and serve as vouchers for the DNA sequences.

For the bird hosts, we amplified the nuclear beta fibrinogen intron 7 (FIB7) gene and portions of the mitochondrial cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) genes using PCR. Primers and reaction conditions for FIB7 and cyt *b* were described by Johnson and Clayton (2000). We amplified a portion of the COI gene using the primers L6625 and H7005 (Hafner et al., 1994), and we used the same primers to amplify COI for *Columbicola*. For lice, we also amplified a portion of the nuclear elongation factor 1 alpha (EF-1α) gene using the primers EF1-For 3 and EF1-Cho10 (Danforth and Ji, 1998). We amplified the mitochondrial 12S ribosomal RNA (12S) gene using the primers 12Sai and 12Sbi (Simon et al., 1994). We sequenced avian and louse PCR products as described by Johnson and Clayton (2000) and Johnson et al. (2001a). Sequences for the protein-coding genes were aligned using Sequencher (GeneCodes). Very few indels were present in FIB7, so alignment by eye was straightforward. Sequences of the 12S gene for *Columbicola* were aligned according to a secondary structure model of this gene region for lice and other insects (Page, 2000; Page et al., 2002). Gaps were treated as missing character states. All sequences were submitted to GenBank (accessions AF29704–AF279743, AF278608–AF278643, AY151003–AY151026).

Phylogenetic Analyses

Unless otherwise indicated, all phylogenetic analyses were performed using PAUP* (Swofford, 2001). To compare phylogenetic signal between gene regions, we used the partition homogeneity test (Farris et al., 1994, 1995; Swofford, 2001). We analyzed each gene region independently and in a combined analysis. For tree reconstruction, we first used parsimony with all characters equally weighted and unordered and then used TBR branch swapping with 100 heuristic random addition replicates. The tree for Columbiformes was rooted on small New World ground doves, as indicated by Johnson and Clayton (2000). We used *Oxylipeurus chiniri* as an outgroup for the analysis of *Columbicola* (Cruickshank et al., 2001). We bootstrapped parsimony trees using 1,000 heuristic search replicates (Felsenstein, 1985).

As an alternative method of phylogeny reconstruction and to evaluate the sensitivity of tree topology to phylogenetic method, we used maximum likelihood (ML). For each data set, we estimated the simplest model that could not be rejected in favor of a more complex model according to the framework of Huelsenbeck and Crandall (1997) as implemented in ModelTest (Posada and Crandall, 1998). To evaluate relative support for various branches in the likelihood trees, we used bootstrapping with 100 replicates involving random addition with TBR branch swapping.

Cophylogenetic Analyses

To compare host and parasite trees, we first used reconciliation analysis (Page, 1990a) as implemented in the program TreeMap 1.0 (Page, 1995). We randomized the parasite tree with respect to the host tree to determine whether more cospeciation could be inferred than expected by chance (Page, 1990b). We also used a data-based parsimony method (Johnson et al., 2001c) to test whether significant incongruence could be detected between host and parasite data sets. This method takes into account phylogenetic uncertainty in both the host and parasite trees in evaluating whether there is statistical support for incongruent regions of the trees. Using simple reconciliation analysis (i.e., no host switching), we determined the number of ancestral parasite species that would have occurred on ancestral hosts if no host switching had occurred. Modern columbiform birds generally have one or two or at most four species of *Columbicola* occurring on them (only one case of four species on a single host species; Clayton and Price, 1999). Thus, assuming that it is unlikely that ancestral hosts would have more sympatric species of parasites occurring on them, we used a reconstruction of more than four sympatric parasite species as evidence that host switching is necessary to explain current host–parasite distributions rather than only duplication and sorting events. Increasing the number of host-switching events tends to reduce the number of inferred "sympatric" parasite lineages.

Reconciliation analysis by itself does not allow for host switching. Jungles analysis (Charleston, 1998) implemented in TreeMap 2.0 (Charleston and Page, 2001) allows for cospeciation, duplication, sorting events, and host switching. Costs can be assigned to these various events, and host-switching events are constrained to occur among contemporary nodes. Jungles analysis finds the optimal cophylogenetic solution given the event costs and the various constraints enforced. We used costs of 0 for cospeciation and 1 for duplication, sorting, and host-switching events. TreeMap 2.0, which implements Jungles analysis, is still in the experimental stages, and computational intensity is high when the number of host switches is large. Because of these computational problems, we also used TreeFitter (Ronquist, 1998) as an event-based parsimony method that incorporates cospeciation, duplication, sorting events, and host switching. Costs can be assigned to each of these events and an optimal (most parsimonious) solution can be found. TreeFitter estimates the number of cophylogenetic events of each type and tests whether there are more or less of these events than expected by chance. However, TreeFitter does not output the cophylogenetic scenarios, so the results cannot be directly interpreted in the framework of a historical scenario. For TreeFitter analyses, we set the cost of cospeciation to 0 and the costs of duplication and sorting to 1. The cost of host switching

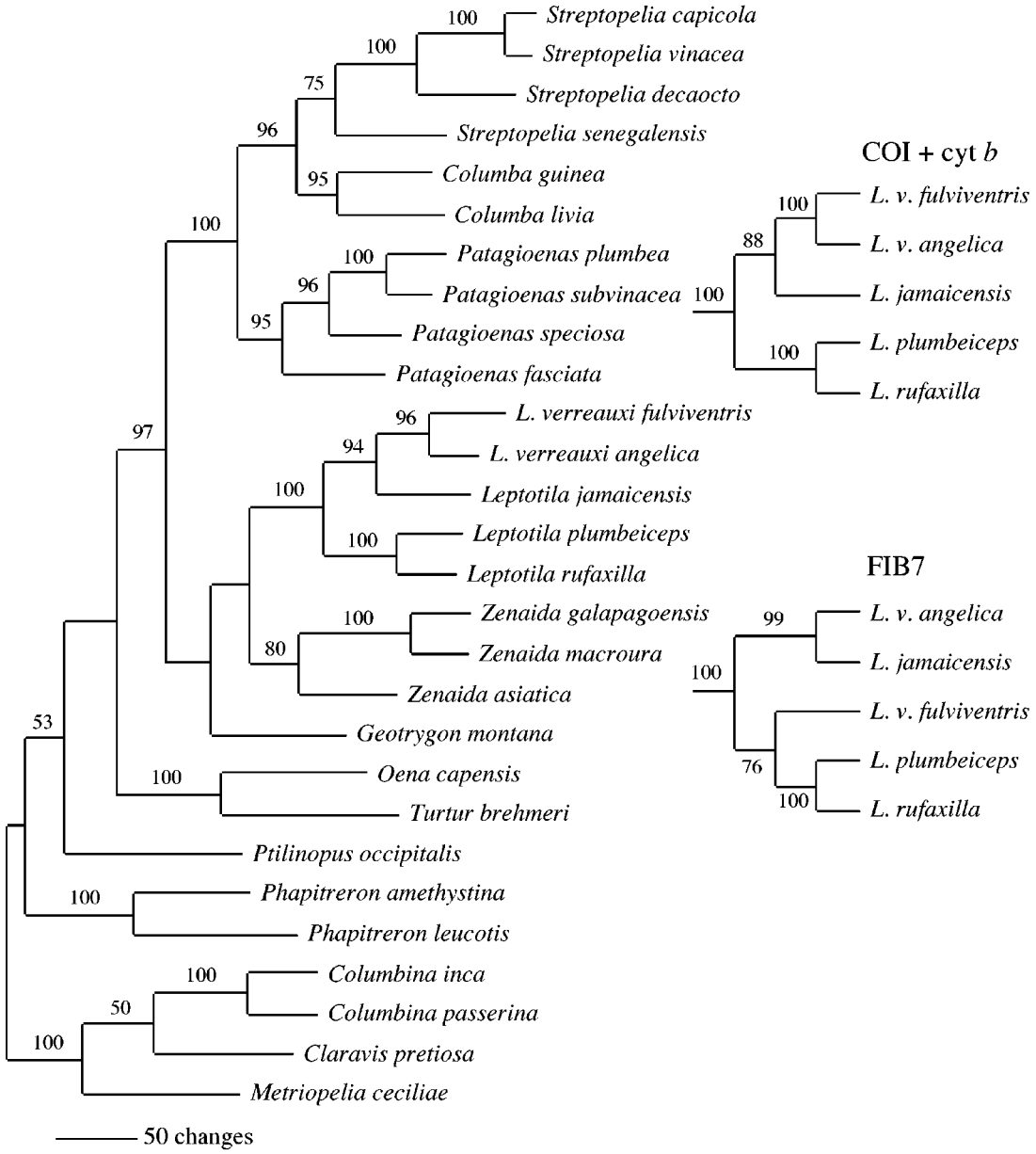

FIGURE 2. Single most-parsimonious tree (length = 2,652, rescaled consistency index = 0.223) for Columbiformes derived from analysis of combined FIB7, cyt *b*, and COI gene sequences. Incongruence between mitochondrial and nuclear gene regions was detected and localized to rearrangements within the genus *Leptotila* (see insets). Numbers above branches indicate support from 1,000 bootstrap replicates. Bootstrap values <50% are not shown.

ranged between 1 and 10. In addition, we randomized the parasite tree to evaluate whether there were greater or fewer of each of the events (cospeciation, duplication, sorting, host switching) than expected by chance. To evaluate the sensitivity of this analysis to uncertainties in the parasite tree, we constructed 100 parsimony bootstrap replicates and compared these 100 trees to the host tree using TreeFitter.

We also evaluated the relative frequency of various cophylogenetic events by examining the associated parasites of terminal host sister taxa. Comparisons of terminal congeneric sister taxa remove the difficulties of inferring events in the distant past of host–parasite lineages. For Columbiformes, there are eight terminal congeneric sister pairs in our analyses. We evaluated whether the lice associated with these terminal congeneric sister pairs showed cospeciation, failure to speciate, or some other combination of events causing incongruence such as sorting or host switching. Terminal duplication events can be recognized as sister taxa of lice on the same host species. For each of the 28 host taxa, we evaluated whether there had been a recent

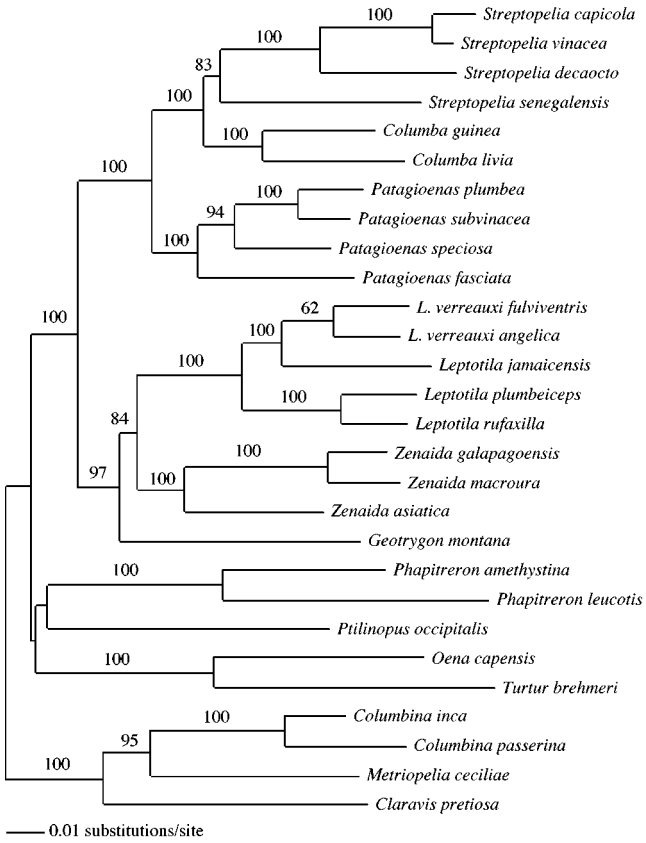

FIGURE 3. Tree derived from maximum likelihood analysis of combined FIB7, cyt *b*, and COI sequences for Columbiformes (L = 15,870.5). Model parameters included general time reversible substitutions (A-C = 1.22; A-G = 7.57; A-T = 0.88, C-G = 0.64; C-T = 10.28; G-T = 1.00), unequal base frequencies (A = 0.290; C = 0.292; G = 0.156; T = 0.262), invariant sites (0.486), and rate heterogeneity according to a gamma distribution (shape parameter = 0.65). Numbers above branches indicate support from 100 bootstrap replicates. Bootstrap values <50% are not shown.

duplication event in their associated lice by determining whether any host species had multiple louse taxa that are sisters.

Because we sequenced a homologous gene region for both birds and lice, we evaluated the relative rate of molecular evolution in hosts and parasites by comparing branch lengths involving cospeciated nodes (copath analysis; Page, 1994). To determine which nodes are consistent with an underlying history of cospeciation, we used the taxon-deletion method of Johnson et al. (2001c). The branches in the tree consistent with strict cospeciation were then used in copath analysis to estimate relative substitution rates. Copath lengths were estimated under both parsimony (number of changes) and ML (estimated branch lengths under a model selected by likelihood ratio tests in ModelTest).

RESULTS

Phylogenetic Analyses

Aligned sequences of FIB7, cyt *b*, and COI for Columbiforms resulted in a data set of 2,625 base pairs. Aligned sequences for EF-1α, COI, and 12S for *Columbicola* totaled 1,170 base pairs. Partition homogeneity test analysis comparing mitochondrial and nuclear genes for the pigeons and doves resulted in significant incongruence ($P = 0.01$). However, this incongruence could be attributed to a strongly supported difference in position for a single taxon, *Leptotila jamaicensis*. A partition homogeneity test of the nuclear versus mitochondrial data without this species in the analysis was not significant ($P = 0.16$). The mitochondrial data set supported (bootstrap = 100) monophyly of *L. verreauxi*, but analysis of the nuclear intron (FIB7) resulted in strongly supported (bootstrap = 99) paraphyly of *L. verreauxi* with respect to *L. jamaicensis* (Fig. 2). Simulation studies (Barker and Lutzoni, 2002; Darlu and Lecointre, 2002) indicated that when rates between data sets vary dramatically, the partition homogeneity test can be significant even though there is not a difference in the underlying phylogeny. Although mitochondrial rates are about five times greater than nuclear rates in doves (Johnson and Clayton, 2000), this difference is generally not high enough to cause a significant result alone (Barker and Lutzoni, 2002) and probably is due to differences in sorting between nuclear alleles and mitochondrial alleles in the genus *Leptotila* (K. P. Johnson and J. D. Weckstein, unpubl. data). Because the difference in the placement of

L. jamaicensis does not dramatically affect the conclusions based on these analyses, we conducted further analyses combining the nuclear and mitochondrial data sets.

Combined parsimony analysis of FIB7, cyt *b*, and COI data for doves resulted in a single tree (Fig. 2). This tree was generally well supported by bootstrap replicates; 21 of 25 nodes had bootstrap support >75%. Likelihood ratio tests of the combined data set suggested that a general time reversible model incorporating uneven base frequencies, invariant sites, and heterogeneous rates according to a gamma distribution (GTR + I + G) could be supported by the data over simpler models. ML analyses using these parameters produced a single most likely tree (Fig. 3) that was nearly identical to the parsimony tree. Differences involved a few weakly supported nodes near the base of the tree.

A partition homogeneity test of the three genes for the *Columbicola* data set did not reveal significant heterogeneity ($P = 0.33$). Even though rates among these three genes vary dramatically, a situation for type I error (Barker and Lutzoni, 2002; Johnson et al., 2002a, in press), we did not detect significance. Thus, we combined the data sets in subsequent analyses. Parsimony analyses of the combined EF-1α, COI, and 12S data sets for *Columbicola* produced a single tree, which was completely resolved (Fig. 4). The species groups identified by Adams (2002) and represented in our data set were all monophyletic, which included the *extinctus*, *columbae*, *gracilicapitis*, and *passerinae* species groups. In this tree, 11 of 19 nodes were supported in >50% of bootstrap replicates.

Likelihood ratio tests indicated that a model incorporating four transversion rates and a single transition rate, unequal base frequencies, invariant sites, and rate heterogeneity according to a gamma distribution (TVM + I + G) was supported over simpler models. Likelihood searches with the estimated model parameters recovered a single most likely tree (Fig. 5). This tree was identical in most respects to the parsimony tree, but a few of the deeper, weakly supported branches were rearranged.

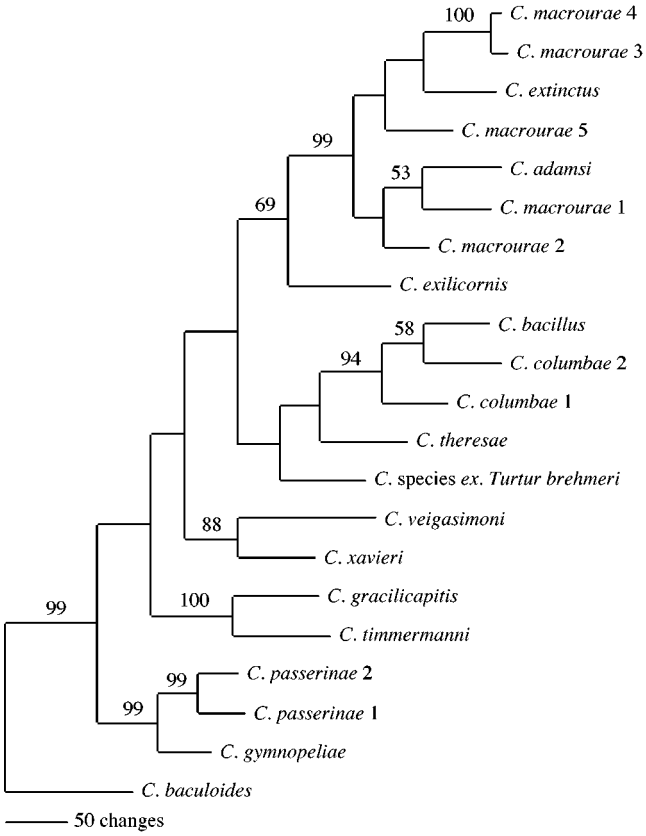

FIGURE 4. Single most-parsimonious tree (length = 2,205, rescaled consistency index = 0.180) for *Columbicola* derived from analysis of combined EF-1α, 12S, and COI gene sequences. Tree was rooted on *Oxylipeurus chiniri* (not shown). Numbers above branches indicate support from 1,000 bootstrap replicates. Bootstrap values <50% are not shown.

Cophylogenetic Analyses

Reconciliation analysis (Page, 1990a) of the parsimony trees for Columbiformes and *Columbicola* identified 9 potential cospeciation events and required 11 duplications and 61 sorting events to explain the differences in host and parasite phylogenies (Fig. 6). Nine cospeciation events is more than would be expected by chance ($P = 0.03$). Reconciliation analysis does not allow for host switching. Some ancestral hosts were reconstructed as having five species of *Columbicola*. We consider this reconstruction unlikely, because modern hosts have one, two, or at most four species of *Columbicola*. We used Jungles analysis in TreeMap 2.0 to examine whether allowing host switching would reduce the number of lice reconstructed to be on ancestral hosts. With costs of 0 for cospeciation and 1 for host switching, duplication, and sorting events, we found optimal reconstructions for one, two, and three host-switching events. However, we were not able to find solutions involving more than three host switches because of the extreme computational complexity. The optimization costs declined dramatically as the number of host switches was increased (Table 2), so the optimal number of host switches is likely to be higher than three. For one, two, and three host switches, five louse species were reconstructed on some ancestral hosts, so reconstructions that allow for a maximum of four species of lice on an ancestral host are likely to involve more than three host switches.

TreeFitter analysis uses a different optimization criterion, termed event-based parsimony (Ronquist, 1998).

TABLE 2. Costs of Jungles optimizations[a] for speciation events in *Columbicola*.

No. host switches allowed	Cospeciation	Duplication	Sorting	Optimization cost
0	22 (20)	18 (20)	53 (54)	71 (74)
1	24 (20)	16 (20)	47 (48)	64 (69)
2	24 (22)	16 (18)	41 (41)	59 (61)
3	26 (22)	14 (14)	34 (36)	51 (57)

[a]Numbers in parentheses indicate results from comparison of maximum likelihood trees.

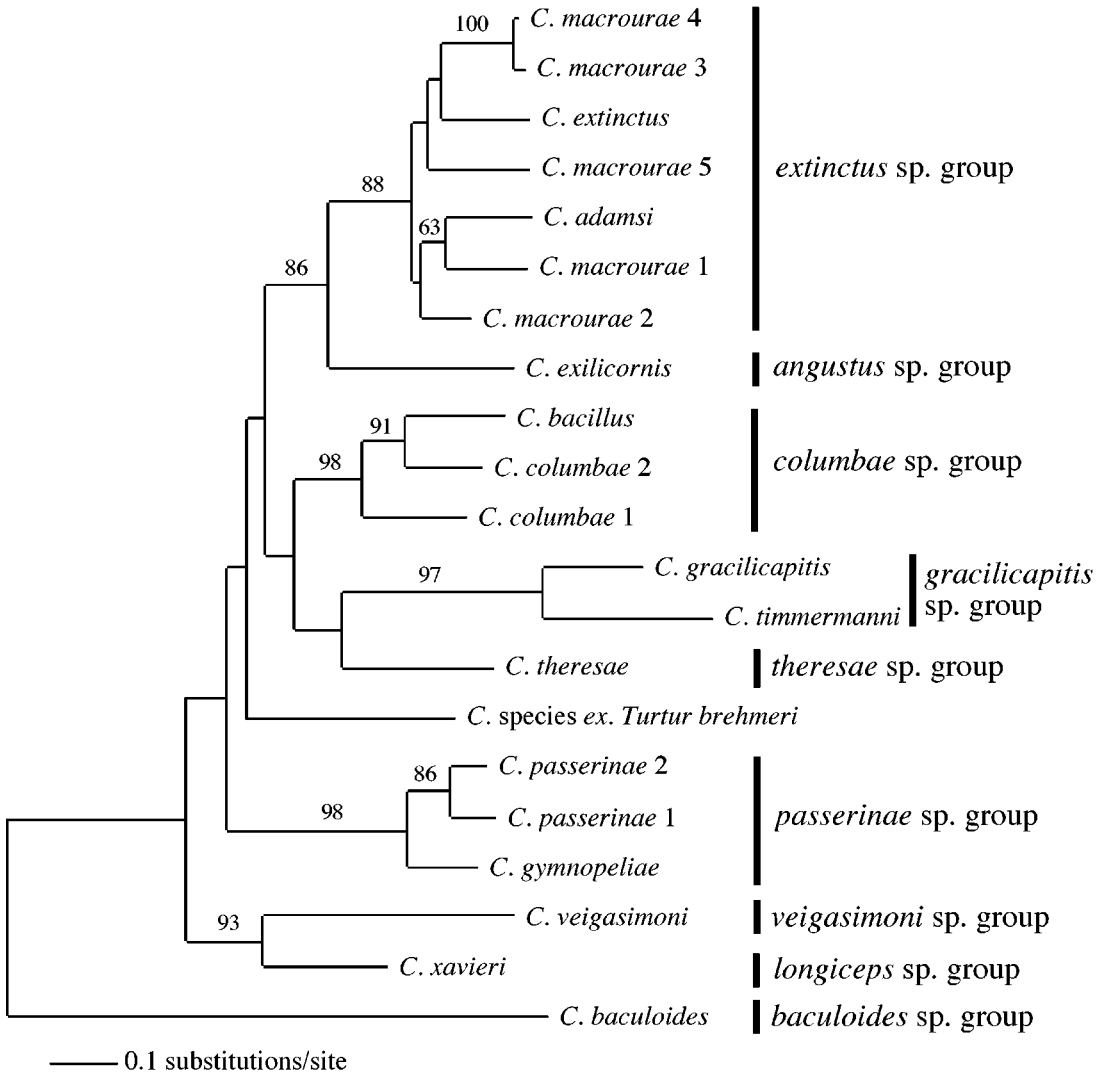

FIGURE 5. Tree derived from maximum likelihood analysis of combined EF-1α, 12S, and COI gene sequences for *Columbicola* (L = 10094.9). Model parameters included four transversion rates and a single transition rate (A-C = 0.86; A-G = 8.67; A-T = 1.11; C-G = 2.40; C-T = 8.67; G-T = 1.00), unequal base frequencies (A = 0.322; C = 0.160; G = 0.178; T = 0.341), invariant sites (0.386), and rate heterogeneity according to a gamma distribution (shape parameter = 0.46). Tree was rooted on *Oxylipeurus chiniri* (not shown). Numbers above branches indicate support from 100 bootstrap replicates. Bootstrap values <50% are not shown. Species groups recognized by Adams (2002) are indicated by bars.

Because this algorithm is computationally simpler than Jungles analysis, reconstructions involving many host switches can be recovered. The disadvantage of TreeFitter analysis is that the phylogenetic placement of the events cannot be visualized or output. The results of increasing host-switching costs from 1 to 10 (Table 3) spanned the range of explanations for the incongruence between host and parasite trees: from nearly complete switching (cost 1) to complete duplication and sorting (costs 9 and 10). For low host-switching costs, the number of cospeciation events was always more than expected by chance (or nearly so for cost 2, $P = 0.08$). The number of host-switching events was often lower than expected by chance when the parasite tree was randomized with respect to the host tree. Comparing 100 boostrap parasite trees with the host tree in TreeFitter analyses produced a similar pattern (Table 4). The number of cospeciation events was generally higher than expected by chance, whereas the number of host switches was often lower than expected by chance, especially at low host-switching costs. The numbers of duplication and sorting events were generally the same as expected by chance.

Comparison of the ML trees (Figs. 3, 5) with reconciliation analyses recovered eight cospeciation events, a number not significantly different from that expected by chance ($P = 0.088$). However, several of the TreeFitter analyses (Table 3), which allows host switching, indicated a significant ($P < 0.05$) or nearly significant ($P < 0.10$) degree of cospeciation. The number of cospeciation events was also lower for Jungles analysis of the ML trees than for the parsimony trees (Table 2). However, the general trend of decreasing optimization

TABLE 3. Results of TreeFitter analysis[a] of speciation events in *Columbicola*.

Switching cost	Cospeciation	Duplication	Sorting	Switching
1	8[b] (7–8[b])	0 (0)	1 (1–2)	12[c] (12–13[c])
2	8 (8)	0 (0)	1 (2)	12 (12)
3	10–11[b] (10[b])	0 (0)	6–9 (7)	9–10[c] (10[c])
4	11–12[b] (10)	0–1 (0–1)	9–13 (7–10)	8–9 (9–10)
5	12–13[b] (11)	3–4 (4)	24–33 (25)	3–5[c] (5)
6	13[b] (11)	4 (4–7)	33 (25–40)	3 (2–5)
7	13[b] (11)	4 (7)	33 (40)	3 (2)
8	11–13[b] (10–11)	4–9 (7–10)	33–52 (40–53)	0–3[c] (0–2)
9	11 (10)	9 (10)	52 (53)	0 (0)
10	11 (10)	9 (10)	52 (53)	0 (0)

[a]Numbers in parentheses indicate results from comparison of maximum likelihood trees.
[b]Significantly ($P < 0.05$) more events than expected by chance under costs given.
[c]Significantly ($P < 0.05$) fewer events than expected by chance under costs given.

TABLE 4. Results of TreeFitter analysis[a] of 100 bootstrap replicate *Columbicola* trees.

Switching cost	Cospeciation	Duplication	Sorting	Switching
1	6–11 (99%[b])	0	1–4 (7%[b])	9–13 (99%[c])
2	7–13 (94%[b])	0	1–8 (2%[b])	8–13 (88%[c])
3	8–13 (92%[b])	0–1	1–13 (1%[b])	6–12 (80%[c])
4	9–14 (86%[b])	0–2	3–18	4–12 (52%[c])
5	8–14 (75%[b])	0–6 (6%[b])	5–40 (7%[b])	1–10 (48%[c])
6	10–14 (75%[b])	0–9 (13%[b])	8–54 (7%[b])	0–10 (57%[c])
7	9–14 (75%[b])	1–9 (5%[b])	18–66 (1%[b])	0–7 (47%[c])
8	9–14 (60%[b])	2–10 (1%[c])	20–66	0–5 (36%[c])
9	9–14 (39%[b])	3–11	26–66 (4%[c])	0–5 (7%[c])
10	9–13 (39%[b])	6–11 (1%[c])	30–66 (10%[c])	0–2

[a]Values indicate range of number of events reconstructed across all 100 bootstrap replicates. Percentages in parentheses indicate the fraction of bootstrap replicates that produced a significant result in the direction indicated.
[b]Significantly ($P < 0.05$) more events than expected by chance under costs given.
[c]Significantly ($P < 0.05$) fewer events than expected by chance under costs given.

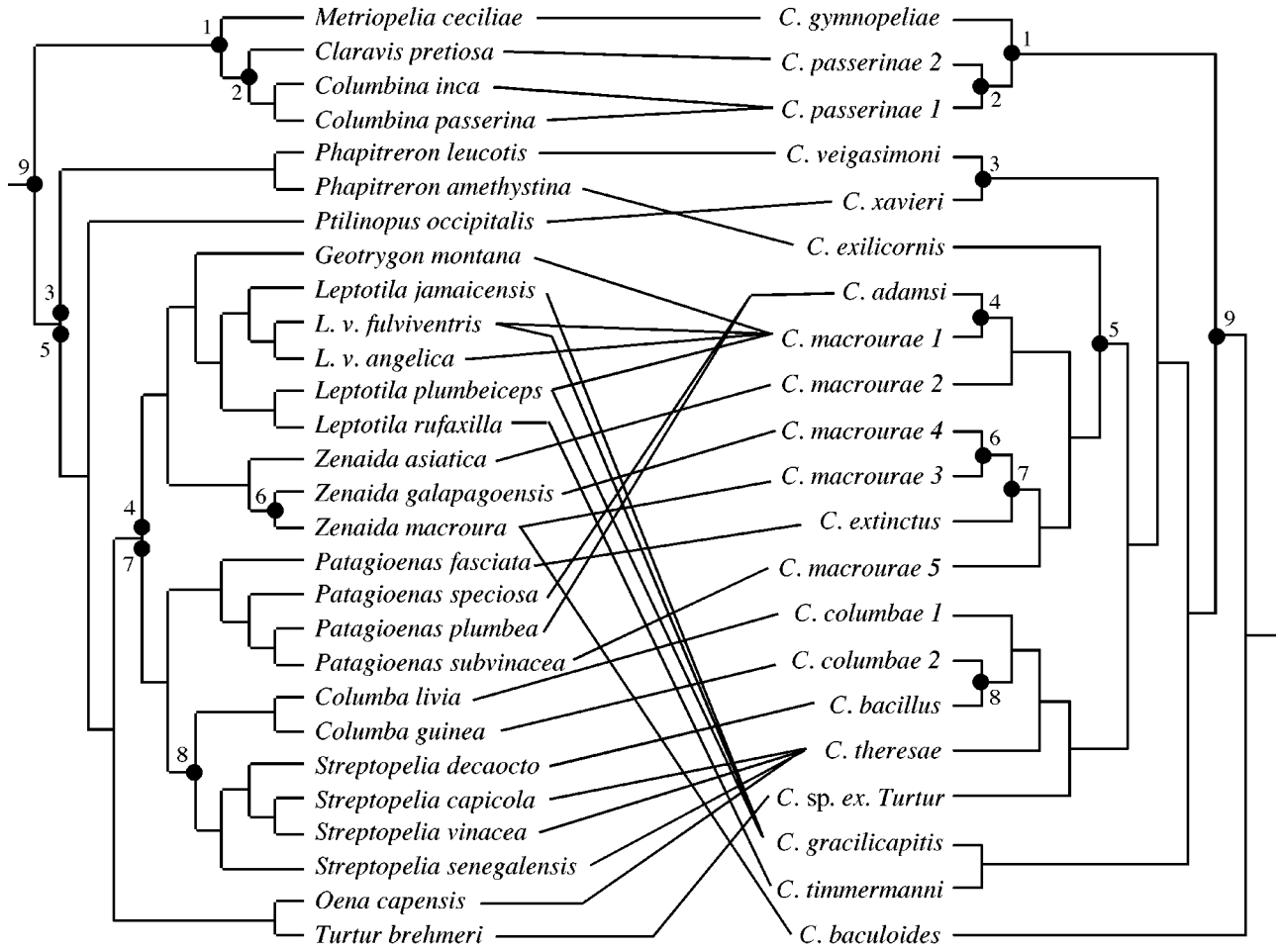

FIGURE 6. Comparison of parsimony trees for columbiform hosts and *Columbicola* parasites. Lines connecting taxa indicate host–parasite associations. Solid circles at nodes are cospeciation events inferred from reconciliation analysis. Numbers indicate corresponding inferred cospeciation events in host and parasite trees. The number of cospeciation events (nine) was significantly higher than expected by chance in randomizations of the parasite tree ($P = 0.03$).

cost with increasing number of host switches was still apparent.

A partition homogeneity test comparing the bird and louse data, using the method described by Johnson et al. (2001c), indicated that the two data sets were not consistent with perfect cospeciation ($P < 0.001$). This result still held when cases were removed in which a parasite was found on multiple hosts and in which a host harbored multiple parasite species ($P < 0.001$). Not until 13 of 31 associations were removed from the data set were the Columbiform and *Columbicola* data sets consistent with complete cospeciation ($P = 0.158$). This result indicates there is considerable significant incongruence between host and parasite trees, even though there also is a significant degree of cospeciation. Generally, the associations removed were either those involving nonspecific lice or those in which there was conflict in bootstrap values between host and parasite trees. This method takes into account uncertainties in the phylogeny as estimated from the sequence data. The branches from the tree resulting from parsimony analysis of this data set (minus cases where a single louse species was found on host sister taxa) were used as copaths for the estimation of relative rates.

Sequences for the COI gene were common to both the bird and louse data sets. We used the copaths tree to reconstruct branch lengths for the COI gene, using both parsimony and likelihood. Major axis regression of parasite versus host parsimony branch lengths estimated a relative rate of 1.61. However, this value is probably an underestimate, because parsimony underestimates the number of multiple substitutions, especially for quickly evolving, highly homoplastic sites. Major axis regression of branch lengths estimated by ML models resulted in a relative rate estimate of 11.07, indicating that substitutions in the COI gene are occurring much more rapidly in lice than in birds.

Eight comparisons of terminal congeneric host sister taxa and associated lice indicated several different patterns (Table 5). Only one terminal cospeciation event was evident (between *Zenaida macroura* and *Z. galapagoensis* and associated lice). In three cases, lice failed to speciate on terminal sister pairs of hosts. In four cases, some combination of duplication, sorting, or host switching was needed to explain the lice associated with congeneric sister taxa. In no case did we find an example of a host species harboring sister taxa of lice. For hosts with multiple louse species, these lice are often in distantly related clades. This result suggests that duplication probably is a rare event in this system.

TABLE 5. Cophylogenetic events estimated from terminal congeneric sister Columbiform taxa and their associated lice.

Event	Number[a]
Cospeciation	1 (1)
Duplication	0 (0)
Failure to speciate	3 (3)
Other incongruence events[b]	4 (4)

[a]Numbers in parentheses indicate results from comparison of maximum likelihood trees.

[b]Combination of host switching, duplication, and sorting.

DISCUSSION

Phylogenetic analyses of one nuclear and two mitochondrial genes for Columbiform birds and their associated feather lice produced well resolved trees for each group. Comparisons of these trees revealed evidence for a significant degree of cospeciation. These data sets also contained significant evidence for incongruence between host and parasite phylogenies, indicating that some combination of duplication, sorting events, and host switching were likely involved in the evolutionary history of these birds and their lice. Estimates of the relative rates of substitution in the mitochondrial COI gene for lice versus birds indicated a substantially elevated rate in lice, consistent with previous studies of louse–vertebrate systems (Hafner et al., 1994; Page et al., 1998).

As implemented in current analytical methods, incongruence between host and parasite phylogenies is explained by a combination of duplication, sorting events, and host switching. More recent methods can give more or less weight to each of these events when deriving an optimal solution. However, the relative importance of each of these processes in natural systems is largely unknown. Because reconstruction of cophylogenetic events deep in host–parasite history can be an uncertain process, one solution is to examine very recently speciated hosts and to evaluate the status of their associated parasites. We found no cases of recent duplication. Sorting events also appear to be rare; we know of only one example of a pigeon or dove that has been well-sampled for lice that lacks *Columbicola*: the New Zealand Pigeon (Paterson et al., 1999). Thus, reconstruction methods applied to *Columbicola* should probably assign a low cost to host switching and relatively higher costs to duplication. In addition, duplication events produce a number of sympatric species on the same host lineage. Information about modern parasite diversity on single host species can be used to evaluate how reasonable particular reconstruction histories might be. In the sampling for our study, we found at most two species of *Columbicola* on a single species of bird, whereas reconstructions involving low numbers of host-switching events (0–3) posit five coexisting species of lice on a single host ancestor. Based on this evidence, host switching is a likely explanation for much of the incongruence between host and parasite trees.

Role of Failure to Speciate

Many cophylogenetic methods (e.g., Jungles, TreeFitter) have difficulty dealing with widespread (i.e., nonspecific) parasites. For example, TreeFitter assumes these widespread parasites represent recent host-switching events. However, the cost of these events is not included in the total cost for a particular cophylogenetic reconstruction. Jungles analysis as implemented in TreeMap 2.0 suffers from a similar problem and arbitrarily

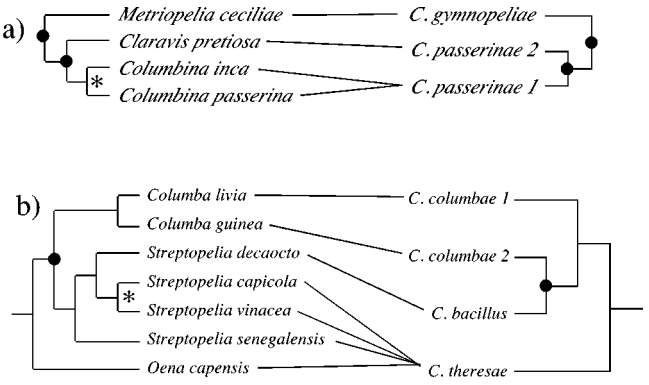

FIGURE 7. Comparisons of parsimony trees in two regions of inferred failure to speciate in *Columbicola*. Nodes at which a failure to speciate is inferred are indicated by an asterisk. a) Small ground doves. *Columbina inca* and *C. passerina* are sympatric congeners but are not true sister taxa (Johnson and Clayton, 2000). b) Turtle-doves and allies. *Streptopelia capicola* and *S. vinacea* are allopatric congeners and true sister taxa (Johnson et al., 2001b).

excludes the additional host–parasite associations. Examination of host–parasite associations and phylogeny for terminal host taxa in our study revealed an important pattern not explored in previous reconstruction methods for host and parasite phylogenies. In the bird louse trees, there are three cases where the lice of recently speciated birds (congeneric sister species) have not speciated.

Speciation of hosts in the absence of speciation of parasites is not currently an event accounted for by cophylogenetic reconstruction methods. Thus, it is important to identify both the frequency of this event in host–parasite coevolutionary histories and the factors promoting a lack of speciation in parasites. A parasite is likely to fail to speciate when it can maintain gene flow among diverging host populations. Because lice are relatively immobile, this process should be unlikely in cases where host speciation is allopatric. In one of the three cases (genus *Columbina*), the host species under consideration are sympatric (Fig. 7a). However, the species included in our analysis, *Columbina inca* and *C. passerina*, are not immediate sister taxa (Johnson and Clayton, 2000). Samples of lice from other relatives closer to each host species were not available. Thus, *C. inca* and *C. passerina* share a species of louse possibly because that louse species has switched off of one host onto the other in sympatry and maintains ongoing gene flow (incomplete host switching, Fig. 1f).

Although the distribution of lice on *Columbina* might not reflect an actual failure to speciate event, the other two cases of failure to speciate involve true allopatric sister taxa. *Streptopelia vinacea* and *S. capicola* are allopatric sister taxa; *S. vinacea* occurs in northwestern subsaharan Africa, and *S. capicola* occurs in southeastern subsaharan Africa. These two species are each other's closest living relatives (Johnson et al., 2001b). *Columbicola theresae* occurs on both of these allopatric sister species and has failed to speciate when these hosts speciated (Fig. 7b). However, *C. theresae* also occurs on other species of doves in Africa, *S. senegalensis* and *Oena capensis*, which are broadly sympatric with both *S. vinacea* and *S. capicola*. Thus, as long as *C. theresae* can disperse among sympatric species, general geographic dispersal by *S. senegalensis* and *O. capensis* may be enough to facilitate gene flow of lice between *S. vinacea* and *S. capicola*. The widespread species of doves may in a sense serve as a vector with respect to *S. vinacea* and *S. capicola*. The other case of failure to speciate also involves a louse (*Columbicola macrourae* 1) that is widespread on other hosts that are sympatric with both allopatric sister taxa.

Failure to speciate in a host-specific louse in the face of allopatric speciation of its hosts seems unlikely, and we can provide no evidence of this process in dove lice. However, a louse species may be able to maintain gene flow among allopatrically speciating species provided there are other sympatric hosts that the louse species utilizes. The role of failure to speciate in the coevolutionary history of host–parasite associations remains an open question. Systems in which parasites have higher gene flow than their hosts are known (Dybdahl and Lively, 1996), and in these systems failure to speciate might be an important process. Future modifications of existing methods for reconstructing host–parasite histories should consider including failure to speciate as a potentially important process.

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