

Seabird and Louse Coevolution: Complex Histories Revealed by 12S rRNA Sequences and Reconciliation Analyses

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Abstract.—We investigated the coevolutionary history of seabirds (orders Procellariiformes and Sphenisciformes) and their lice (order Phthiraptera). Independent trees were produced for the seabirds (tree derived from 12S ribosomal RNA, isoenzyme, and behavioral data) and their lice (trees derived from 12S rRNA data). Brook's parsimony analysis (BPA) supported a general history of cospeciation (consistency index = 0.84, retention index = 0.81). We inferred that the homoplasy in the BPA was caused by one intrahost speciation, one potential host-switching, and eight or nine sorting events. Using reconciliation analysis, we quantified the cost of fitting the louse tree onto the seabird tree. The reconciled trees postulated one host-switching, nine cospeciation, three or four intrahost speciation, and 11 to 14 sorting events. The number of cospeciation events was significantly more than would be expected from chance alone ($P < 0.01$). The sequence data were used to test for rate heterogeneity for both seabirds and lice. Neither data set displayed significant rate heterogeneity. An examination of the codivergent nodes revealed that seabirds and lice have cospeciated synchronously and that lice have evolved at ~5.5 times the rate of seabirds. The degree of sequence divergence supported some of the postulated intrahost speciation events (e.g., *Halipeurus* predated the evolution of their present hosts). The sequence data also supported some of the postulated host-switching events. These results demonstrate the value of sequence data and reconciliation analyses in unraveling complex histories between hosts and their parasites. [BPA; cospeciation; host-switching; lice; Procellariiformes; reconciliation analysis; relative rate test; seabirds; sorting events; Sphenisciformes.]

"One other remarkable analogy [of bird alliances] we would notice, and one perhaps by which it has not yet struck ornithologists, [is] to trace the alliance between the various groups [of lice]." (Sir W. Jardine, 1841)

Jardine's observation of a possible parallel between avian and louse relationships is an early example of the long-lasting fascination biologists have felt for the field of host-parasite coevolution (see Klassen, 1992, for an historical survey). Today, evolutionary biologists regard the presence of parasites on their host species as the outcome of two processes: Either parasites may have had an evolutionary association with the ancestors of their hosts and have been passed down through the host lineage (association by descent), or parasite presence may be a result of parasite species switching from an independent host lineage to the present host (association by colonization) (Brooks and McLennan, 1991). Association by descent will often result in cospeciation, which occurs when a parasite lineage speciates in response to the speciation of its host. Recent developments

in phylogenetics now permit rigorous investigation of the extent of cospeciation (Brooks, 1981; Page, 1992).

Most cospeciation studies have tested Fahrenholz's rule (Lyal, 1986; and see Brooks and McLennan, 1991), which states that parasite phylogeny should mirror host phylogeny and predicts that host and parasite phylogenies should match or be congruent if cospeciation has occurred (Brooks, 1985; Brooks and McLennan, 1991; Klassen, 1992). Previous studies have generally found reasonable congruence between host and parasite phylogenies and have thus inferred a relatively high level of cospeciation (e.g., Brooks and Glen, 1982; Hafner and Nadler, 1988; Guegan and Agnese, 1991; Moran and Bauman, 1994). Congruence between the host and parasite trees has generally been assessed either by visual inspection or by Brooks Parsimony Analysis (BPA). A quantitative procedure developed by Brooks (1981) to assess the extent of cospeciation, BPA uses parasites as character states and uses parasite phylogeny as a character-state tree. Parasite information is transformed into additive binary code, analyzed phylogenetically, and

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mapped onto the host cladogram (Brooks and O'Grady, 1989). Homoplasious characters are generally interpreted as the result of either host-switching events or lineage extinction. The better the fit of the additive binary-coded parasite phylogeny, the greater the extent of inferred cospeciation.

A crucial limitation of many cospeciation studies has been the basic assumption that congruence between host and parasite

trees is evidence of a history of cospeciation, whereas incongruence is evidence of host-switching. Neither of these widely held assumptions is necessarily correct. Brooks and McLennan (1991) have commented that congruence between host and parasite may be caused by sequential colonization of hosts, which coincides with host phylogeny (Fig. 1a). It is unclear how prevalent this problem of false congruence is. Conversely,

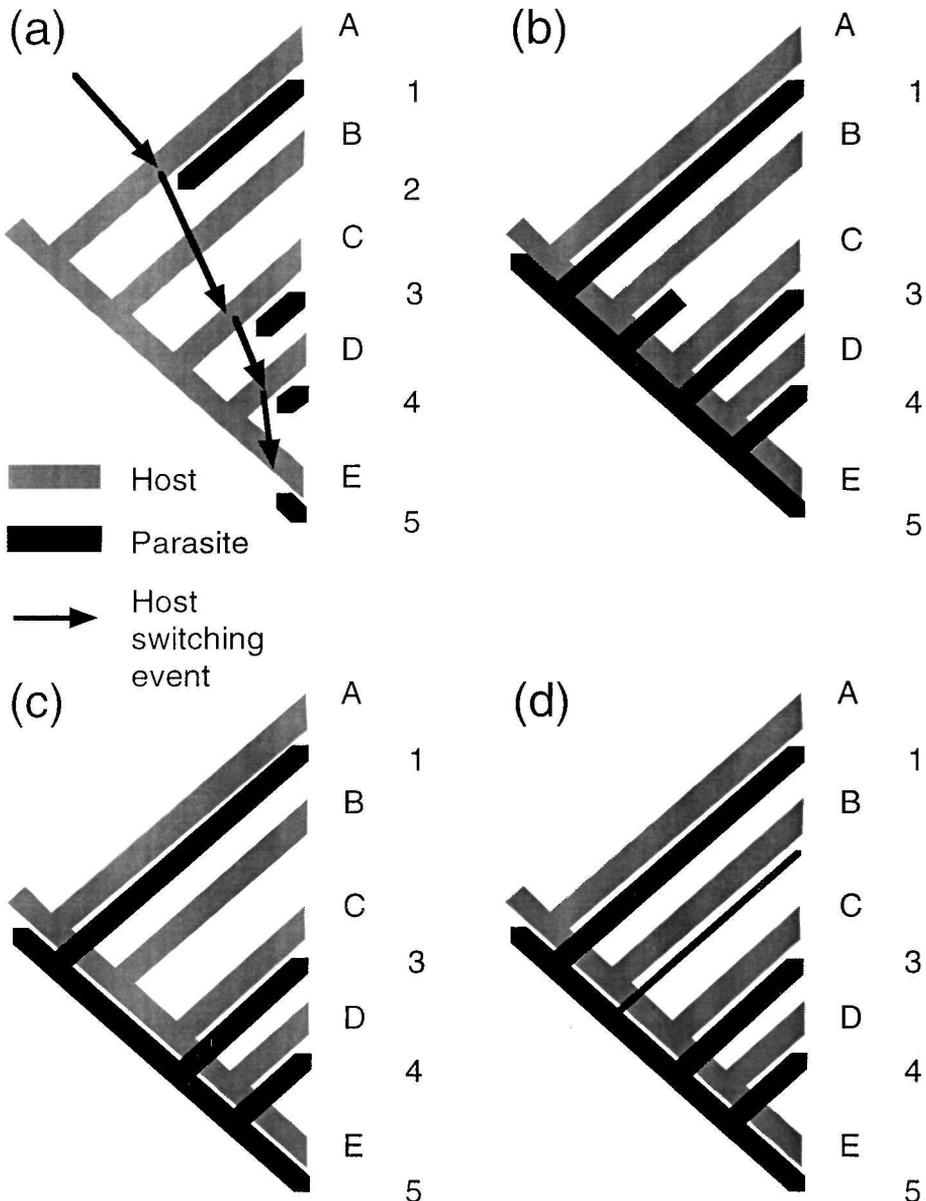


FIGURE 1. Various events may result in the absence of hypothesized parasite 2 from host B. (a) Sequential colonization of hosts (letters) by parasites (numbers) that coincides with the phylogeny of their hosts, but never colonized host B. (b) Extinction of parasite 2 among the ancestors of host B. (c) MTB: The founder population of host B lacked parasites. (d) Parasite 2 is present on host B but has not been detected because of low density or variable distribution.

incongruence may occur between cospeciating host and parasites with intrahost speciation and sorting events that lead to loss of parasites. Intrahost speciation, or redundancy, in conjunction with sorting events (Page, 1992, 1994a), causes false incongruence because the parasite speciates without a corresponding host speciation event. This process results in two or more closely related parasite species inhabiting one host. These parasite species may then both cospeciate with their host, leading to duplicate lineages. The resulting redundancy is analogous to gene duplication (Goodman et al., 1979; Patterson, 1988; Page, 1993a, 1994b), i.e., several different gene/parasite lineages are present in/on one species/host. Parasites may be missing from a particular potential host species because of three types of sorting events: extinction from the ancestral host population (extinction; Fig. 1b), absence from the founder population because of variation in distribution of the ancestral parasite species throughout the ancestral host population ("missing the boat" (MTB); Fig. 1c) (see Paterson and Gray, 1997), or failure to

be detected because the parasite species is in low numbers or is variably distributed in the extant host population (sampling effect; Fig. 1d).

Page (1992, 1993a,b, 1994a,b) developed a method that infers intrahost speciation, cospeciation, sorting, and suspected host-switching events from phylogenetic trees of parasites and their hosts. This method, termed reconciliation analysis, postulates the minimal numbers of intrahost speciation and sorting events needed to reconcile incongruent host and parasite trees without postulating host-switching (Fig. 2). TreeMap is a recent modification to reconciliation analysis that allows host-switching to be addressed in a systematic manner. Using reconciliation analysis, Page (1991, 1993a,b) showed that the coevolutionary history of pocket gophers and their ectoparasitic lice features at least one intrahost speciation event. Reconciliation analysis of chewing lice and seabird hosts (Paterson et al., 1993) inferred as many as 7 intrahost speciation and 32 sorting events. Paterson and Poulin (1999) also used reconciliation analysis to show that parasitic

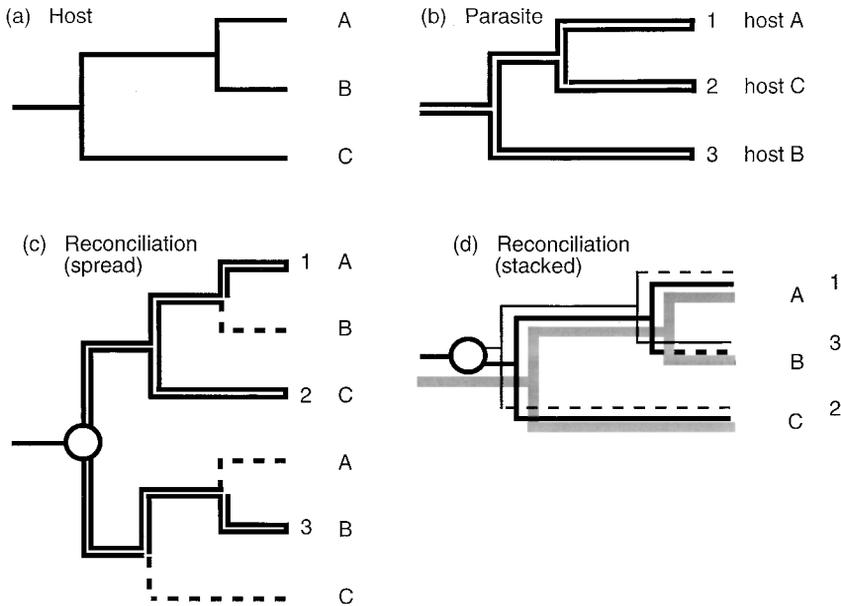


FIGURE 2. Incongruent host (a) and parasite (b) cladograms and the reconciliation tree (c: spread form) and (d: stacked form) of the two. The parasite tree indicates that parasite species 1 and 2 are sister taxa, implying that their hosts, A and C, are most closely related. This conflicts with the host tree, which indicates that species A and B are sister taxa. Reconciliation analysis reconstructs the evolutionary events (cospeciation, intrahost speciation, and sorting events) necessary to produce the observed host and parasite cladograms. Reconciling the host and parasite trees requires one intrahost speciation (open circle) and three sorting events (-). The presence of extant parasites is indicated by solid lines. The shaded branches in (d) represent the host phylogeny and the thin and thick solid lines represent the two parasite lineages. Spread and stacked trees are merely different ways of representing the same information.

copepods have coevolved closely with their marine fish hosts.

In this study we use sequence data and a combination of BPA and TreeMap analysis to investigate the coevolutionary history of seabirds and their phthirapteran chewing lice. Phthirapteran lice are interesting candidates for cospeciation studies. Because they are nonpathogenic, chewing lice can be readily passed down through the seabird's lineage (Clay, 1951; Humphrey-Smith, 1989). Previous studies have suggested that seabirds and their lice follow Fahrenholz's rule (Timmermann, 1952, 1965; Palma and Pilgrim, 1983, 1984; Imber, 1985; Paterson et al., 1993). Ernst Mayr, however, argued that Timmermann displayed an almost "child-like faith" in the ability of seabird lice to reflect their host phylogeny (see Paterson et al., 1995b), and warned that Fahrenholz's rule may be confounded by host-switching and different rates of evolution between host and parasites.

Page et al. (1996) outlined several requirements for a rigorous study of cospeciation. An adequate alpha-taxonomy of both hosts and parasites is essential, especially because closely related parasite species are often very similar morphologically. Reconciliation analysis and BPA use only the topology of trees, so it is thus obviously highly desirable that these trees are accurate. Exhaustive sampling of clades will tend to give stronger tests of hypotheses of cospeciation than will sampling from a range of different clades. Quantitative comparisons of host and parasite phylogenies make possible explicit statistical tests of cospeciation rather than reliance on qualitative assessments. Molecular phylogenies based on homologous genes are extremely useful for cospeciation studies if the sequences evolve in an approximately clocklike manner.

In cospeciation studies, clocklike molecular data can be used in at least four ways. First, if cospeciation has occurred in a host-parasite relationship, then one would expect the divergence times from a particular cospeciation event to be approximately contemporaneous (Hafner and Nadler, 1990; Page, 1993a). Generally, the host is expected to speciate first, followed by the parasite—although the cessation of gene flow may cause the parasite to speciate first because it has more generations per year. The finding of contemporaneous divergence between cospeciating taxa provides an additional,

more stringent, test of cospeciation (Hafner et al., 1994; Hafner and Page, 1995). Second, molecular data can be used to test between interpretations within an analysis. For example, if a parasite has switched hosts, then the genetic distance between the parasite and its sister taxon will be less than the genetic distance between their hosts (Page et al., 1996). Third, molecular data can be used to test between interpretations from different analyses. For example, if reconciliation analysis and BPA conflict in their inferences about specific coevolutionary events (e.g., intrahost speciation or host-switching) then the relative distances postulated by each interpretation can be used to discriminate between them (Hafner and Page, 1995). Fourth, if cospeciation is found, inferences can be made about the factors influencing the comparative rates of molecular evolution in the host and parasite taxa (Hafner et al., 1994; Hafner and Page, 1995).

In an earlier study (Paterson et al., 1993; Paterson and Gray, 1997), we used component and reconciliation analysis to reconstruct the coevolutionary history of 18 seabird species and 19 louse genera, based on a morphological tree for the lice. We found evidence for several intrahost speciation and many sorting events but little evidence for host-switching. In this study we use 12S ribosomal RNA (rRNA) sequence data and reconciliation analysis to examine a subset of the seabirds and their lice that were studied by Paterson et al. (1993). The use of sequence data allows us to test the predictions made by our earlier studies that the coevolutionary history between sea-birds and their lice consists of cospeciation, intrahost speciation, and sorting events with little host-switching. This is the second time that molecular data from both birds and their lice have been used to investigate cospeciation and other coevolutionary events and represents only the third such data set for hosts and parasites in general (see Hafner et al., 1994, Page et al., 1998).

MATERIALS AND METHODS

Seabird Data

The 11 species chosen represent a cross-section of procellariiform and sphenisciform genera found in the New Zealand region. We used the gull *Larus dominicanus* (order Charadriiformes) as an outgroup for the seabirds throughout. These three bird orders

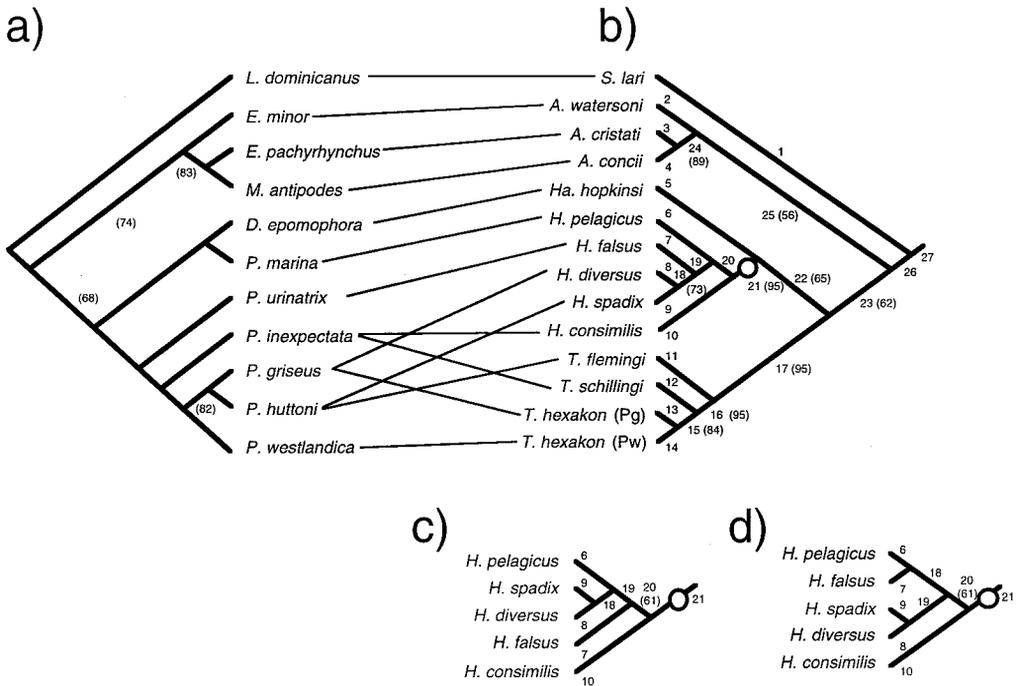


FIGURE 3. (a) Pruned seabird subtree from the total evidence tree of Paterson et al. (1995a; their Fig. 6a). See Table 1 for common names. (b–d) Three maximum likelihood trees generated from louse 12S sequences. Each branch is numbered for BPA. The percentage of times that a branch appears in 10,000 bootstrap replications is recorded in parentheses along each branch (only values >50% are recorded).

are thought to be relatively closely related (Cracraft, 1981; Sibley and Ahlquist, 1990; McKittrick, 1991). Phylogenetic relationships of the seabird species (Fig. 3a) are taken from Paterson et al. (1995a; their Fig. 6a, total evidence tree) and are derived from behavioral and life history, mitochondrial 12S rRNA, and isoenzyme data. The datasets were combined by pooling the behavior (unordered), 12S sequence (equally weighted), and isoen-

zyme (loci as multistate characters) data to produce a total evidence tree (Kluge, 1989; Bull et al., 1993).

Louse Data

Samples of live lice (order Phthiraptera) representing six genera and 14 species were collected from their seabird hosts at the hosts' colonies (Table 1). Lice were removed

TABLE 1. Lice used in this study, their hosts, and collection sites.

Louse species	Host	Collection localities (southern New Zealand)
<i>Austrogonioides concii</i>	<i>Megadyptes antipodes</i>	Otago Peninsula
<i>A. cristati</i>	<i>Eudyptes pachyrhynchus</i>	Jackson Bay, Westcoast
<i>A. watersoni</i>	<i>Eudyptula minor</i>	Green Island, Otago
<i>Halipeurus consimilis</i>	<i>Pterodroma inexpectata</i>	Codfish Island, Stewart Island
<i>H. diversus</i>	<i>Puffinus griseus</i>	Codfish Island, Stewart Island
<i>H. falsus pacificus</i>	<i>Pelecanoides urinatrix</i>	Stephen's Island, Cook's Strait
<i>H. pelagicus</i>	<i>Pelagodroma marina</i>	Motonau Island, Canterbury
<i>H. spadix</i>	<i>Pu. huttoni</i>	Seaward Kaikoura Mts, Marlborough
<i>Harrisoniella hopkinsi</i>	<i>Diomedea epomophora</i>	Otago Peninsula
<i>Saemundsonia lari</i>	<i>Larus dominicanus</i>	Dunedin, Otago
<i>Trabeculus flemingi</i>	<i>Pu. huttoni</i>	Seaward Kaikoura Mts, Marlborough
<i>T. hexakon</i>	<i>Pu. griseus</i>	Codfish Island, Stewart Island
	<i>Pr. westlandica</i>	Punakaiki, Westcoast
	<i>Pt. inexpectata</i>	Codfish Island, Stewart Island

from seabirds by using forceps and were kept at -80°C . Heads were removed and stored in ethanol for identification; the remaining parts were used for isoenzyme analysis and DNA extraction. Molecular data were obtained from the 12S rRNA gene by polymerase chain reaction with use of the "universal" primers (Kocher et al., 1989), followed by DNA sequencing (Sanger et al., 1977). Sequences reported in this paper have been placed in EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under accession numbers Y14909–Y14924. The alignment is available on the *Systematic Biology* Web site (<http://www.utexas.edu/depts/systbiol>).

The great variation among louse sequences demanded a critical assessment of the nature of these sequences. We built secondary structures for all of the sequences based on other compilations of small sub-unit rRNAs (Hickson et al., 1996). We found satisfactory stem (31–42) and loop regions for the majority of the molecule. Stems 45, 47, and 48 were more difficult to construct in some of the species, so we omitted them from the analysis. To assess the identity of the sequences, we used the FASTA algorithm (Pearson and Lipman, 1988) of the GCG package (Devereux et al., 1990). This algorithm allows for indels and is thus an appropriate sequence comparison program for noncoding genes such as 12S. One randomly selected *Halipeurus* species (*Halipeurus spadix*) and one randomly selected *Trabeculus* (*Trabeculus flemingi*) sequence were compared with all the sequences deposited in the GenBank and EMBL databases. The 12S sequence data were analyzed using PAUP* 4.0d64 (Swofford, 1999). Maximum likelihood (ML) was used as the optimality criterion. An heuristic search with TBR branch swapping and 10 random sequence additions was conducted. The general time reversible (GTR) model (Yang, 1994) was used to estimate the ML tree. Both the rate and the among-site rate heterogeneity parameters were estimated during the search. We used *Saemundssoniana lari* as the outgroup. Bootstrap analysis (Felsenstein, 1985), using the neighbor-joining algorithm with 10000 replications, was conducted to obtain some measure of the robustness of the data. This analysis used maximum likelihood distances derived from the GTR model with the parameters set to the values estimated for the optimal tree(s). To assess whether any incongruence between

the louse tree(s) and the host tree could be due merely to sampling error, we used the Kishino–Hasegawa (1989) test in PAUP* 4.0d64. We constrained a louse tree to be maximally congruent to the seabird tree and compared the differences in likelihood scores between the constrained and optimal trees, using the GTR model and parameters estimated above. The Kishino–Hasegawa test was one-tailed.

Brook's Parsimony Analysis

BPA has been used for the last 20 years to examine cospeciation between host and parasite. This protocol developed by Brooks (1981) uses parasites as character states and parasite phylogeny as a character-state tree. Parasite information is transformed into additive binary code, analyzed phylogenetically, and mapped onto the host cladogram (Brooks and O'Grady, 1989). Homoplasious characters may be interpreted as being the result of either host-switching events or intra-host speciation followed by sorting events. A BPA of the louse phylogeny and host distribution was conducted.

Reconciliation Analysis

A contrasting approach to reconstructing cospeciation between host and parasite is reconciliation analysis. Reconciliation analysis of seabird and louse trees was performed using TreeMap (Page, 1994a), which examines all possible host-switching scenarios (with accompanying sorting and duplication events) and selects the outcome that maximizes the number of cospeciation events between host and parasite trees. The maximum number of cospeciation events is then assessed for significance against a distribution of cospeciation events derived from random host and parasite trees.

Host-switching may also be detected by analyzing duplication events. Duplication events imply that intrahost speciation events by parasites, or host-switching events, or inaccuracy of host or parasite phylogenies—singly or in various combinations—are responsible for the patterns observed. To distinguish between the competing explanations for duplications, one has to make a decision on the relative likelihood of the events occurring. For instance, one intrahost speciation and three sorting events may be equivalent to postulating one host-switching

event. This is an empirical question that requires further study. Molecular data are likely to be valuable in resolving these questions in which relative divergence times support one scenario over the other.

Rates of Evolution

If hosts and parasites evolve at a uniform and constant rate (which may differ for the two groups), assessment of relative times of divergence is simplified. Evidence of rate heterogeneity within seabird and lice sequences was assessed by using the relative-rate test (Sarich and Wilson, 1973; Li and Graur, 1991). The number of substitutions between sequences (d) was calculated by using the GTR maximum likelihood model. The shortest trees for both seabirds and lice were tested for rate heterogeneity. If no rate heterogeneity is found, then the existence of local molecular clocks for seabird and louse phylogenies is supported. Given the existence of a local molecular clock, the host-parasite trees can then legitimately be remodeled as dendrograms, where the heights of the clusters represent estimated divergence times (Page, 1991; Hafner and Page, 1995). Page (1991, 1993a) has suggested that only common clusters between host and parasite trees should be compared. Our study used the following steps:

1. Test for homogeneous rates of nucleotide substitution within the trees.
2. If both host and parasite trees are homogeneous, then regress d values of cospeciating or codivergent parasites against the corresponding host pairs. The regression slope will give the rate difference (R) between parasite and host sequences (do parasite genes evolve at the same rate?), and the intercept on the y -axis will give the relative timing of cospeciation events (do hosts always speciate first?) (Hafner and Page, 1995). Reduced major-axis regression is the most appropriate analysis because of errors in the estimates of both x and y variables (see McArdle, 1988).
3. Rescale louse d (d/R) so that louse and seabird d values are directly comparable. Postulated coevolutionary events such as host-switching and intrahost speciation events can then be assessed. For example, if an intrahost speciation event is postulated, then the rescaled d values between

the parasites involved will be equal to or greater than the d values of their hosts; if the rescaled d values are significantly less than the hosts' d values, then a host-switching event is implied.

RESULTS

Louse Variation

We compared our sequences with a consensus sequence drawn from 184 species (including chordate, arthropod, annelid, crustacean, mollusc, onychophoran, echinoderm, nematode, actinozoan) (Hickson et al., 1996). At the primary sequence level, several highly conserved regions are faithfully represented in our sequences: UGGCgG in stem 32; YAGAG in stem 33; the first of the two U-G pairs in stem 34; the terminal C-G pair of stem 35; the C two bases before the start of stem 35'; a CY at the base of stem 36; the Y bulge, ACc, and GUC in stem 38; the A before stem 39; the AGuCA before stem 38'; the RG at the end of stem 36'; the U in stem 45; the GAA before stem 33'; and the GUA after stem 33'. A few highly conserved regions are, however, apparently absent—most notably, the YUACA motif at the end of stem 34', which is YYUCW in *Halipeurus*, *Trabeculus*, and *Harrisoniella*, and quite different in *Austrogoniodes* and *Saemundssonina*. At the secondary structure level, the base-pairing is very clear: Stem 33 has five bases pairing in every sequence, stem 34 shows at least 10 bonds in all sequences (including G-U pairs), stem 36 shows Watson-Crick bonding at six or seven positions in every sequence, and stem 42 has at least four bonded pairs (including a few G-U pairs) in every sequence. However, stem 38 includes some A-C pairs and lacks the conserved U-A and A-U pairs. At a more gross level, all three *Austrogoniodes* sequences contain a 35- to 40-bp insertion that may represent a single 5- to 7-bp stem and have loop 37 between stems 36 and 38; two sequences possess a 59-bp insertion after stem 39'. These insertions parallel those referred to in the alignment analysis of *Mytilus edulis* (Hickson et al., 1996).

What are we to make of these complex data? Besides the louse species used in the study, we were not able to amplify 12S from the genera *Austromenopon*, *Docophoroides*, *Episbates*, and *Paraclisis*. This variable success is to be expected if the gene has a fast rate of evolution in lice. We do not believe

that many, if any, of the unusual features we observed can be ascribed to sequencing errors. The repeatability and general phylogenetic consistency within genera argue that the majority of the novelties observed are in fact real. Could we be looking at nuclear genes? Once again, the consistency within genera (e.g., the two large insertions in all three *Austrogoniodes*) and consistent novelty in some conserved motifs (e.g., YUACA) argues against this interpretation: It would demand that we have consistently amplified copies at the expense of the real 12S. Moreover, we would not expect to observe consistently the many highly conserved motifs if we were looking at copies. For the same reasons, contamination does not seem likely, and anyway the sequences were repeatable. We can rule out any host sequence because there were no matches with data from birds. Functional nuclear RNAs are far too conserved to be considered contenders. No louse 12S sequences are available elsewhere for comparison with ours. Sequences from the louse species *Halipeurus spadix* and *Trabeculus flemingi* were compared with all sequences deposited in the GenBank and EMBL databases by using the FASTA algorithm of the GCG package. In both cases the five closest sequences were from insect 12S rRNA. *Halipeurus spadix* had a 62.5% similarity with *Drosophila melanogaster* 12S (accession no. X97155), and *Trabeculus flemingi* had 63.3% similarity with *Mantis religiosa* 12S (accession no. U17792). The match between the lice and other insect sequences could be improved substantially by manual realignment. The overall good match with 12S from other insects confirms that our sequences are from lice. The explanation we favor for the highly divergent lice sequences is that lice are simply unusual. We will report and discuss these findings more fully elsewhere.

Seabird and Louse Trees

The seabird phylogeny derived from combined 12S sequence, isoenzyme, and behavior and life history data by Paterson et al. (1995a; their Fig. 6a) was pruned to represent the 11 species used in this study (Fig. 3a). Maximum likelihood analysis of the louse species mitochondrial 12S ribosomal RNA gene (423 bp, three regions of ambiguous alignment: 114–165, 211–285, 350–374 were excluded) found three trees (Fig. 3b–d: Ln

likelihood = 2082.32). As might be expected, given the relatively short length of the louse sequences, the bootstrap values were not consistently high. The fragility of the louse tree obviously limits the confidence we can have in any interpretation that is based on these trees. However, the louse tree indicated that each genus used in this study was monophyletic. The Kishino–Hasegawa test indicated that a tree constrained to be maximally congruent with the seabird phylogeny was significantly different to the optimal trees ($-\ln$ likelihood difference = 27.80, $t = 2.58$, $P < 0.05$).

Trabeculus hexakon was collected from *Puffinus griseus* and *Procellaria westlandica* and showed a high level of variation for intraspecific nucleotide substitutions. *Trabeculus hexakon* from the two populations differed at 53 nucleotide sites, of which 23 were transversions. This amount of variation was greater than some other interspecific differences; e.g., *H. spadix* and *H. diversus* differed at 39 nucleotide positions, of which 7 were transversions. The extent of differentiation between populations of *T. hexakon* on *Puffinus griseus* and *Procellaria westlandica* may warrant a further investigation of their species status.

BPA

Four most-parsimonious host cladograms (consistency index (CI) = 0.84, retention index (RI) = 0.81) were derived from the BPA data (Table 2). There were several points of incongruence in the BPA cladogram relative to the seabird tree, such as *Procellaria westlandica* as sister taxon to *Pterodroma inexpectata* (because both taxa shared *Trabeculus hexakon*, see Fig. 4). To reconstruct this coevolutionary relationship, we optimized the recorded louse trees (Fig. 3b–d) onto the seabird tree (Fig. 3a). The optimizations confirmed that the distribution of louse lineages were consistent with a close history of coevolution with seabirds (Fig. 4). The tree 1 louse characters mapped onto the tree with a minimum of eight sorting events and one episode that required a more complex explanation (Fig. 4a: character 20). The distribution of character 20 indicates that further explanation is required to explain the distribution of *H. pelagicus* and *H. consimilis*. For example, character 20 implies two scenarios: (1) Either the ancestor of *H. consimilis* was present

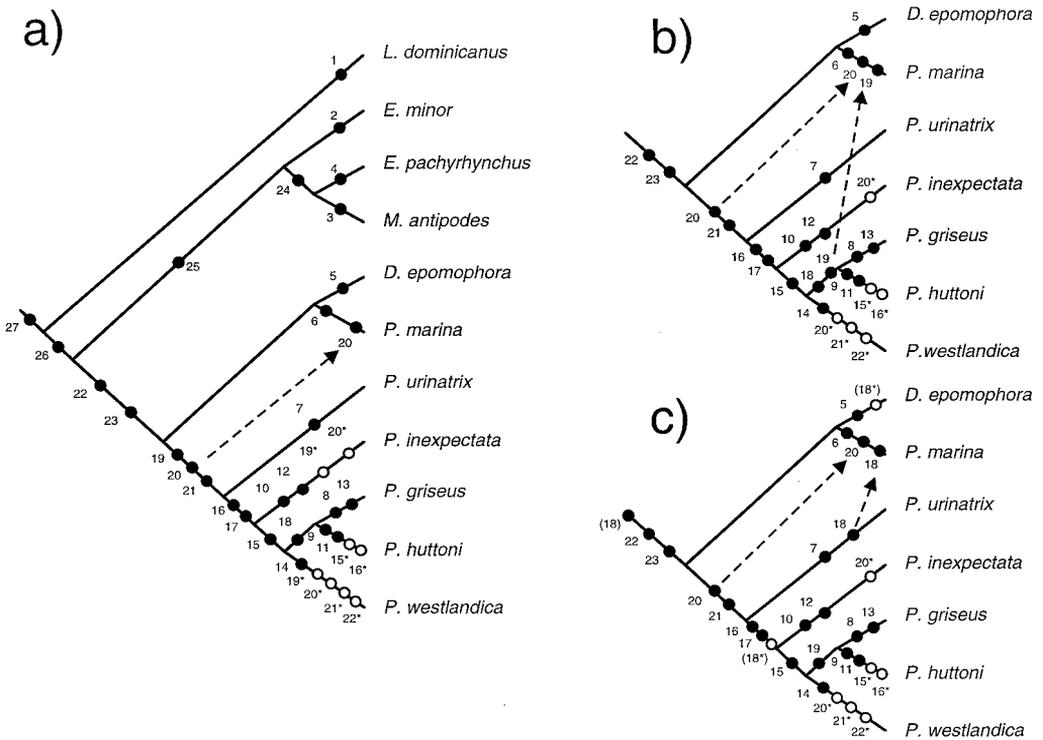


FIGURE 4. Additive binary codes of the louse trees (Fig. 3; a = Fig. 3b, b = Fig. 3c, c = Fig. 3d) optimized onto the seabird tree. Gains (closed circles) and losses (open circles and stars) are illustrated. Potential host-switching or intrahost speciation events are shown (arrows).

on the ancestor of the petrels but has been lost in the albatrosses, shearwaters, and diving petrels, or (2) there has been a host-switching event between storm petrels and pterodromid petrels. The tree 2 and 3 louse characters mapped onto the tree with a minimum of six sorting events and two episodes that required a more complex explanation (Fig. 4b: characters 19 and 20; Fig. 4c: characters 18 and 20). The distribution of character 19 in tree 2 indicates that further explanation is required to explain the distribution of *H. falsus* relative to the other *Halipeurus* species. For example, character 19 implies one of two scenarios: either the ancestor of *H. falsus* was present on the ancestor of the procellariid petrels but the lineage has been lost in the pterodromid and *Procellaria* petrels, or there has been a host-switching event between shearwaters and diving petrels.

Reconciliation Analysis

The TreeMap analyses of the shortest louse and seabird trees (Fig. 5, tree 1: [cospeciation]

9, [duplication] 4, [host switch] 0, [sorting] 14; tree 2: 9, 3, 1, 11; tree 3: 9, 3, 1, 11) showed that there were significantly more cospeciation events than expected by chance (all $P < 0.01$). All three reconciled trees hypothesized a coevolutionary history of seabirds and lice that involved at least 26 or 27 evolutionary events. TreeMap analysis of louse trees 2 and 3 indicated that allowing one host-switching event to occur (*H. pelagicus* switching from a pterodromid ancestor to the storm petrels, which corresponds to events 18 and 19 in the BPA [Fig. 4]) increased the maximum number of cospeciation events to 9, or equal to that in tree 1. An alternative to this scenario is that the position of *H. pelagicus* as sister taxon to *H. consimilis* is inaccurate. For example, a change from (*H. falsus*((*H. pelagicus* *H. consimilis*))(*H. diversus* *H. spadix*))) to (*H. pelagicus*(*H. falsus* (*H. consimilis*(*H. diversus* *H. spadix*)))) would also give 9 cospeciation events with no host-switching event required. Some evidence suggests there is little confidence in the topology of this part of the tree (Fig. 3b: bootstrap values of 50% and less).

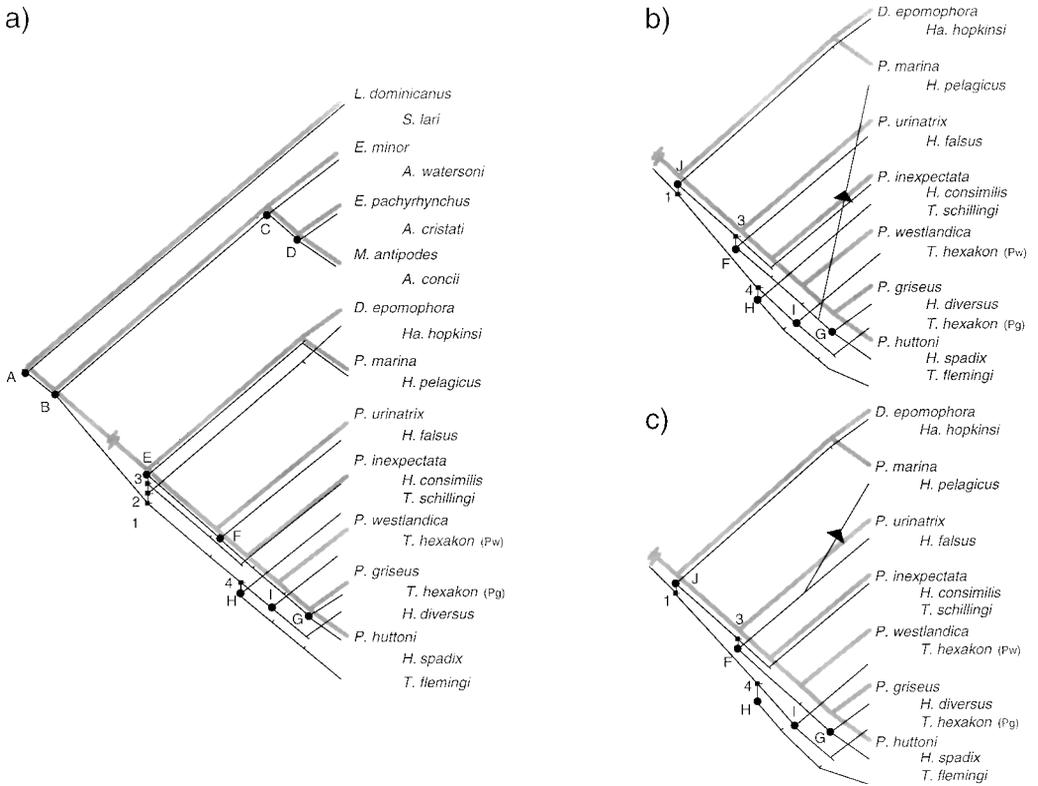


FIGURE 5. Stacked TreeMap reconciliation of seabird (Fig. 3a) and louse phylogenies (a = Fig. 3b; b = Fig. 3c; c = Fig. 3d). Louse relationships are mapped onto the seabird phylogeny and postulated evolutionary events are indicated: cospeciation (A–L), sorting events (short branches), intrahost speciation or duplications (1–4). The thick line represents the seabird phylogeny, and the louse genera are represented by thin lines. Potential host-switching events are indicated with arrows.

Ad hoc analysis of duplication events also provides evidence about host-switching. For example, the most basal duplication (Fig. 5: event 1) postulates that an intrahost speciation event occurred along the preceding branch, indicating that the procellariiform ancestor hosted at least two lineages of lice: one that led to *Halipeurus* and *Harrisoniella*, and the other that led to *Trabeculus* (Fig. 6). Another example, the duplication involving *Trabeculus* (Fig. 5: event 4) may be interpreted in three ways (Fig. 7). Incongruence may be explained by an intrahost speciation event in the ancestral procellariid of this group in which the two lineages arose, one that led to *T. flemingi* on *Puffinus huttoni* and *T. schillingi* on *Pterodroma inexpectata* but was lost by sorting events on the two shearwaters, and the other that led to *T. hexakon*, being present on the shearwaters but absent on the other two hosts (Fig. 7a). Alternatively, a host-switching event such as the ancestor of *T. flemingi* switching from a

procellariid species, perhaps *Pt. inexpectata*, to *Pu. huttoni* may have occurred (Fig. 7b). Finally, the phylogenetic trees for either the

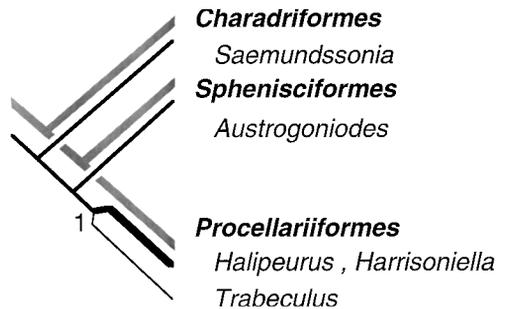


FIGURE 6. Subtree from the seabird–louse reconciliation tree illustrating a duplication event (Fig. 5: 1). The shaded branches indicate host phylogeny; solid lines indicate louse phylogeny. The duplication implies that a louse intrahost speciation event occurred on the ancestors of the petrels, leading to two closely related lineages inhabiting the petrel ancestor.

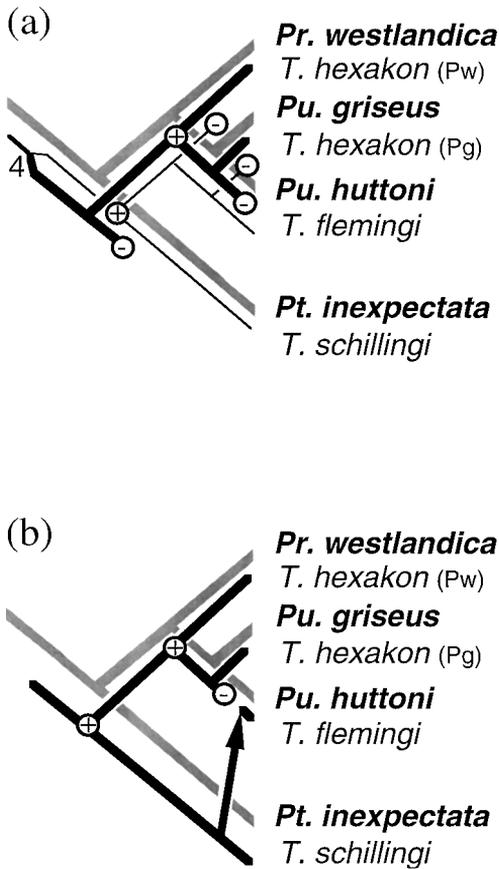


FIGURE 7. Subtree from the seabird–louse reconciliation tree illustrating a duplication event (Fig. 5: 4) involving the *Trabeculus* louse species and their procellariid hosts. The shaded branches indicate host phylogeny; solid lines indicate louse phylogeny. There are at least two alternative explanations for the incongruent duplication: (a) an intrahost speciation event in the ancestors of the procellariid petrels, followed by four sorting events in the two louse lineages; (b) a host-switching event of ancestral *T. flemingi* from a procellariid species to *Puffinus huttoni* in conjunction with one sorting event.

seabirds or lice may be inaccurate. Which of these options is the best interpretation of the incongruent duplication rests on the relative likelihood of each scenario. The first scenario requires five evolutionary events, a single intrahost speciation event plus four sorting events (Fig. 7a). The second scenario requires two evolutionary events, one host-switching and one sorting events (Fig. 7b). The third scenario requires some measure of accuracy of the relationships of the seabirds and lice assessed in this analysis, such as comparison with other phylogenetic results for these taxa; however, there have been no other such published studies for these taxa.

In choosing between the first two scenarios, we must determine whether a host-switching event has the same likelihood as an intrahost speciation event, or we must use molecular information to assess relative divergence times (see below).

Rates of Evolution and Molecular Information

Tests for rate heterogeneity were made for both the seabird and louse shortest trees. Using d values showed that the seabird tree did not significantly differ from the null hypothesis of rate homogeneity. The same was true for the louse tree. A lack of rate heterogeneity among branches is support for the existence of a localized molecular clock. Following Page (1991), we used cluster heights (Table 3) from cospeciation events to investigate relative rates of nucleotide substitution and divergence times. Cospeciation event A was excluded from the analysis because it represents the most basal portions of the seabird and louse trees, and the high level of divergence from other sequences led to this one component unduly biasing the estimate of evolutionary rates. The correlation between the homologous cluster heights of the seabirds and lice was significant (adjusted $r^2 = 0.69$, $P = 0.004$).

The cospeciation events showed a significant correlation with those of their hosts, indicating that these louse and seabird hosts cospeciated at similar relative times. The y -intercept (-0.19) of the line of best fit to the points is not significantly different from zero

TABLE 3. Cluster heights in units of corrected distances (d) for the outgroup and cospeciating nodes between seabirds and chewing lice. Each cluster height is the mean distance between descendent taxa that diverge at that node. For example, the seabird d for component C is the mean of the distance between (1) *E. minor* and *E. pachyrhynchus*, and (2) *E. minor* and *M. antipodes*. The louse d for component C is the mean of the distance between (1) *A. watersoni* and *A. cristati*, and (2) *A. watersoni* and *A. concii*.

Component	Lice	Seabird
A	2.491	0.220
B	1.044	0.211
C	0.210	0.092
D	0.005	0.048
E	0.265	0.177
F	0.145	0.102
G	0.097	0.037
H	0.273	0.093
I	0.216	0.076
J	0.724	0.177

($P = 0.201$). This indicates that the speciation between lice and their seabird hosts has been approximately synchronous (Hafner and Nadler, 1990). The slope of the line of best fit is 5.5, significantly different from 1.0 ($P = 0.004$), indicating that molecular change has been faster in the lice than their hosts (reduced major-axis regression: McArdle, 1988; Plotnick, 1989). This 5.5-fold greater rate of evolution for lice relative to their hosts is similar to results obtained by Hafner et al. (1994) and Moran et al. (1995), who also found that parasites evolve more rapidly than their hosts.

DISCUSSION

Agreement Between the Results of BPA and Reconciliation Analyses

The results of both the BPA and the reconciliation analysis support the idea that the seabird–louse relationship has generally been one of association by descent rather than of association by colonization. Both the BPA and reconciliation analyses also indicated that the coevolutionary relationship was more complex than a simple history of cospeciation. The Kishino–Hasegawa test indicated that the incongruence between seabird and louse trees is not a result of sampling error but requires a more complex explanation, such as host switching, intrahost speciation, and sorting events. Both BPA and reconciliation analyses indicated that sorting events were common and that host-switching episodes have been relatively rare. In comparing the effectiveness of BPA and reconciliation analysis in recovering the same cospeciation history, we note that BPA identifies six to eight sorting events (reconciliation 11–14), one of the four duplication events found by the reconciliation analyses (Fig. 5: event 3), and the potential host-switching event involving *Halipeurus pelagicus* or *H. falsus* that is also found by reconciliation analysis. Both the findings that host-switching is rare and that sorting events are common seem to fit with seabird and louse biology.

Host switching should be relatively rare; there are few seabird interspecific interactions, and lice are unable to survive for long off the host (Wilson, 1934; Nelson and Murray, 1971; Marshall, 1981). Even within a species, opportunities for transfer of lice between hosts are probably limited to trans-

mission from parents to chicks and between partners during nesting and copulation (Timmermann, 1965). Most Procellariiformes (albatrosses, shearwaters, petrels) have little physical contact among individuals either during foraging or within their colonies. Many species burrow, so nests are physically isolated from each other.

If host-switching is likely to be rare, then, conversely, “missing the boat” may be a relatively common event among parasites like phthirapterans because of the patchy nature of louse distribution. Fowler and Price (1987) found that 40% of a population of Wilson’s storm petrel (*Oceanites oceanicus*) carried 0–3 individuals of the louse *Philoceanus robertsi* and that the distribution as a whole fit a negative binomial curve. This agrees with our own observations about the patchiness of lice on seabirds; e.g., only 3 of 12 *Puffinus griseus* sampled carried lice. Of the procellariiform species sampled, none of the 106 individual birds carried all of the louse species found on that species (pers. obs.). Small founder groups of seabirds, therefore, will not be likely to have the full range of lice found on the species as a whole. If this group speciates, some of the louse species will be absent from their hosts. An example of a potential MTB event is that of the effect on diversity of ectoparasite species inhabiting house sparrows (*Passer domesticus*), as has been observed in North America. This introduced population of birds has only 35 of the original 69 ectoparasite species present in the European population (Brown and Wilson, 1975). The house sparrow was also released into New Zealand from Britain, and New Zealand sparrows possess only two of the five louse species found in their parent population (Brown and Wilson, 1975; Pilgrim and Palma, 1982). This pattern of sorting events occurring in daughter populations appears to be fairly general within the New Zealand bird fauna (Paterson et al., 1999).

Interestingly, previous studies (e.g., Page, 1990; Paterson et al., 1993) using tree comparison metrics to assess congruence between host and parasite trees, while confirming a history of cospeciation, provide us with much less information. A congruence approach will not reconstruct coevolutionary events, such as sorting events. Further, such an approach will overestimate the lack of fit between host and parasite trees by confusing incongruence caused by host-switching

with that caused by intrahost speciation and sorting events.

Differences Between BPA and Reconciliation Analyses

Brooks (1996) and Hoberg et al. (1997) have argued that the BPA and reconciliation approaches, although sharing many points in common, have different world-views and explanatory domains arising from their different histories. The striking similarity of the results discussed above suggest that this is overstated; instead, the differences are methodological. The question is, which method produces the most accurate results? Page (1994b) and Paterson and Gray (1997) have claimed that in some situations BPA can produce unrealistic interpretations of the homoplasy found when the parasite tree is mapped onto the host phylogeny. The problem arises from treating each of the additive binary codes for the louse phylogeny as independent. For example, the homoplasy codes 18 and 19 (Fig. 4) correspond to the monophyletic group of *Halipeurus* species, excluding *H. falsus*. The BPA reconstructs these homoplasies as two host-switching events, whereas in the TreeMap analysis they arise from a single event (*H. pelagicus* switches hosts) (Fig. 5). The BPA thus may overestimate host-switching.

Rates of Evolution and Molecular Information

Molecular information allows us to test more than just whether cospeciation events occur synchronously between host and parasites and to calculate rates of evolution. Hypotheses of host-switching inferred by the TreeMap analysis may also be assessed. *Halipeurus pelagicus* is inferred to have host-switched from procellariid petrels to storm petrels. The competing explanations for this host-parasite distribution are either that the common ancestor of both storm petrels and procellariid petrels was host to the ancestor of *H. pelagicus* and that the lineage has been passed down or that the reconstructed relationships are inaccurate. If *H. pelagicus* has host-switched, then this must have occurred after the evolution of the procellariid group and, therefore, the rescaled d value ($d/5.5$) between *H. pelagicus* and *H. consimilis* must be less than the d value between *Pterodroma inexpectata* and the other procel-

lariid petrels ($d = 0.083-0.128$). Alternatively, if the lice have been passed down from a common seabird ancestor, the rescaled d value between *H. pelagicus* and *H. consimilis* must be equal to or greater than the d value between *Pelagodroma marina* and the other procellariid petrels ($d = 0.137-0.203$). That the rescaled d value between *H. pelagicus* and *H. consimilis* equals 0.054 supports a hypothesis of host-switching. The second alternative of an inaccurate topology, however, is also quite possible. The branch placing *H. pelagicus* as the most basal member of this group does not have strong bootstrap support. In summary, there is no support for an intrahost speciation event involving *H. pelagicus*, but there is support for host-switching, an inaccurate topology, or both.

Molecular data also allow the testing of different scenarios involving duplication events postulated by the reconciliation analysis (Fig. 5: events 1-4). For example, Figure 6 illustrates duplication event 1 from Figure 5. This scenario postulates that two louse lineages differentiated before the evolution of the Procellariiformes. If this is the case, we would expect that the rescaled louse distances between the *Halipeurus/Harrisoniella* and *Trabeculus* lineages would be equal to or greater than the distances of the corresponding seabird distances (*Diomedea epomophora* and *Pe. marina* to the other procellariiform species). As Table 4 shows, the range of louse distances overlaps that of the hosts for duplication 1, which indicates support for a duplication event. Duplication event 4 is illustrated in Figure 7 and does not support a duplication event. The duplication and host-switching events identified in the BPA were supported, although other potential duplications were not detected (Fig. 5: events 2 and 3).

TABLE 4. Cluster heights in units of corrected distances (d) for the heights of duplication events (postulated intrahost speciation by lice). Note that the louse d values have been rescaled to compensate for the 5.5-fold increase in the rate of evolution in lice (i.e., $d/5.5$) to directly compare seabird and louse d values.

Duplication	Rescaled louse	Seabird
1	0.139-0.272	0.137-0.211
2	0.118-0.154	0.137-0.211
3	0.033-0.055	0.137-0.211
4	0.046-0.062	0.075-0.076

CONCLUSION

Our results show that the coevolutionary history of seabirds and lice has been punctuated by intrahost speciation, and sorting events but relatively few host-switching events. What causes each of these events to occur on some occasions and not others? Can we predict which lineages are more likely to be prone to intrahost speciation or sorting events? For instance, intrahost speciation events might be expected on hosts with more than one feather type because there would be several niches available for lice to radiate into. Penguins have only one type of feather. The fact that only a single genus, *Austrogoniodes*, is found on all penguin species (except for *Aptenodytes patagonicus*, which has the monotypic *Nesiotinus demersus*), is consistent with the idea that host morphology might influence the likelihood of intrahost speciation events. Louse species with patchy distributions and noncolonial hosts may be more prone to sorting events than would species with a uniform distribution and colonial hosts. A strategy for investigating these types of questions would be to focus the analysis on one genus of seabirds that host lice genera of contrasting distributions. For example, the *Pterodroma* petrels may be a suitable group because they contain species with a range of life histories, from tropical surface nesters to sub-antarctic burrowers, and seven genera of lice.

Reconciliation analysis, in combination with molecular data, offers a promising method for the reconstruction of the coevolutionary history of host and parasites. To date, the use of reconciliation analysis has centered on studying host-parasite cospeciation (Page, 1990, 1991, 1993a,b; Paterson et al., 1995a,b; Hoberg et al., 1997; Paterson and Gray, 1997). Here we have shown the promise that this method has in unraveling complex histories of cospeciation, sorting, intrahost speciation, and host-switching events. We urge those working in the area of coevolution to explore the benefits of reconciliation analysis in other host and associate systems.

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