Syst. Biol. 53(1):111–119, 2004

ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150490264987

Evolutionary Relationships, Cospeciation, and Host Switching in Avian Malaria Parasites

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Abstract.—We used phylogenetic analyses of cytochrome *b* sequences of malaria parasites and their avian hosts to assess the coevolutionary relationships between host and parasite lineages. Many lineages of avian malaria parasites have broad host distributions, which tend to obscure cospeciation events. The hosts of a single parasite or of closely related parasites were nonetheless most frequently recovered from members of the same host taxonomic family, more so than expected by chance. However, global assessments of the relationship between parasite and host phylogenetic trees, using Component and ParaFit, failed to detect significant cospeciation. The event-based approach employed by TreeFitter revealed significant cospeciation and duplication with certain cost assignments for these events, but host switching was consistently more prominent in matching the parasite tree to the host tree. The absence of a global cospeciation signal despite conservative host distribution most likely reflects relatively frequent acquisition of new hosts by individual parasite lineages. Understanding these processes will require a more refined species concept for malaria parasites and more extensive sampling of parasite distributions across hosts. If parasites can disperse between allopatric host populations through alternative hosts, cospeciation may not have a strong influence on the architecture of host–parasite relationships. Rather, parasite speciation may happen more often in conjunction with the acquisition of new hosts followed by divergent selection between host lineages in sympatry. Detailed studies of the phylogeographic distributions of hosts and parasites are needed to characterize these events. [Avian malaria; Component; cospeciation; cytochrome *b*; *Haemoproteus*; host–parasite relationships; ParaFit; phylogeny; *Plasmodium*; speciation; TreeFitter.]

Several cases of cospeciation have been described for parasites with strong vertical transmission between hosts, including lice (Demastes and Hafner, 1993; Hafner and Page, 1995; Page, 1995; Page and Hafner, 1996; Paterson et al., 2000; Clayton et al., 2003) and viruses that spread by direct contact between hosts (McGeoch et al., 2000) and bacterial and viral symbionts that are passed directly from mother to offspring through eggs (Moran and Baumann, 1994; Baumann et al., 1995; Thao et al., 2000; Whitfield, 2002). As tests of cospeciation have been applied more broadly, many cases fail to provide support: e.g., the hantaviruses of *Apodemus* mice (Nemirov et al., 2002), *Wolbachia* and hymenopteran parasites of fig wasps (Shoemaker et al., 2002; Weiblen and Bush, 2002), monogenean worm parasites of fish (Desdevises et al., 2002; Zietara and Lumme, 2002), schistosome parasites of snails (Morgan et al., 2002), and feather lice of birds (Johnson et al., 2002). The evolutionary relationships of parasites that are transmitted by intermediate vectors are less well known, but where vectors are generalists such parasites have increased possibilities of switching between host species. In this study, we analyzed the phylogenetic relationships of malarial parasites and their passerine avian hosts to estimate the relative importance of cospeciation and host switching in a system in which parasites are transmitted by free-living dipteran vectors.

Avian malaria parasites belong to the genera *Plasmodium* and *Haemoproteus* (suborder Haemosporina within the protozoan phylum Apicomplexa) (Atkinson and Van Riper, 1991). These parasites share developmental characteristics related to their life cycle of alternating phases of sexual and asexual reproduction, which require both a vertebrate host and an arthropod vector (Garnham, 1966). The primary vectors for *Haemoproteus* parasites are biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) (Wirth, 1974; Harwood and James, 1979; Kettle, 1982) and louse-flies (Diptera: Hippoboscidae) (Bequaert, 1954; Kettle, 1982). Avian *Plasmodium* is transmitted most commonly by *Culex* mosquitoes (Bequaert, 1954; Forrester et al., 1980; Kettle, 1982; Atkinson and Van Riper, 1991; Telford et al., 1997; Nayar et al., 1998), although other genera of mosquitoes are involved in the transmission of mammalian *Plasmodium*. The parasite genera also differ in that *Plasmodium* undergoes asexual multiplication in the peripheral blood whereas *Haemoproteus* does not. *Plasmodium* is thought to be the more dangerous parasite for birds (Van Riper et al., 1986; Atkinson et al., 2000); *Haemoproteus* is generally more prevalent in host populations but likely has fewer health effects on the host (Atkinson and Van Riper, 1991).

The broad evolutionary relationships among malaria parasites are reasonably well understood (Perkins and Schall, 2002). The genus *Leucocytozoon*, which comprises avian parasites transmitted by simuliid flies, is sister to *Plasmodium* and *Haemoproteus*, according to a cytochrome *b* phylogeny of the group. Mammalian *Plasmodium* is sister to avian and reptilian malaria parasites, and avian *Plasmodium* is either sister to or paraphyletic with respect to *Haemoproteus*, which is restricted to birds and reptiles (Escalante and Ayala, 1994; Escalante et al., 1995; Bensch et al., 2000; Perkins and Schall, 2002; Ricklefs and Fallon, 2002; Waldenström et al., 2002).

At a finer taxonomic scale of host species and parasite lineages, the prevailing idea until recently was that malaria parasite species were host specific, if not to species then at least to host family (Atkinson, 1986; Bennett et al., 1993, 1994). The taxonomy of malaria parasites is based primarily on morphological characters (Coatneyi et al., 1971; Cogswell, 2000), but there has been a strong reliance on host identity as well. Analyses

of DNA sequences of the mitochondrial cytochrome *b* gene-coding region are beginning to reveal a more complex picture featuring more limited correspondence between parasite and host phylogeny. For example, among 68 lineages of avian malaria parasites, Ricklefs and Fallon (2002) recovered one parasite lineage from avian hosts in different families (Passeridae and Pycnonotidae), seven from hosts in confamilial genera, and two from different hosts in the same genus. Bensch et al. (2000) found a malaria parasite of tits (*Parus*: family Paridae) nested within a lineage of parasites of Old World warblers (family Sylviidae), and Waldenström et al. (2002) reported considerable sharing of parasite lineages among hosts within avian families, including one case of a parasite shared by species in the families Sylviidae and Ploceidae. Ricklefs and Fallon (2002) also examined hosts of 17 closely related malaria parasite lineages (average cytochrome *b* sequence divergence of 1.2% ± 1.1%) and found seven pairs in the same host genus, an additional six pairs in the same family, and four pairs from different families (Paridae–Parulidae; Turdidae–Parulidae; Mimidae–Parulidae; Corvidae–Turdidae). In spite of substantial evidence of host switching, the significantly nonrandom distribution of closely related parasite lineages among avian families also suggests a general conservatism of host distribution based on coevolution between malaria parasites and their hosts.

Ricklefs and Fallon (2002) sampled parasite lineages sparsely across the entire breadth of the phylogeny of avian hosts and from sites on five continents. This large-scale but shallow sampling of taxonomically and geographically isolated hosts may have created some of the close associations observed between hosts and their parasites. In this study, we reexamined cospeciation and host switching in avian malaria parasites with more robust sampling of a single avian clade (primarily forest dwelling songbirds: order Passeriformes) within one region (eastern North America and the West Indies). This more focused analysis provided increased sensitivity to coevolutionary relationships in avian malaria parasites. We used a number of standard tree-based approaches to assess the match between parasite and host phylogenies.

Materials and Methods

Parasite haplotypes identified in this study were based on 350 base pairs (bp) of the malaria cytochrome *b* gene. Most of the parasites were recovered from the Lesser Antilles and southern Missouri, with smaller numbers of additional samples from elsewhere in the West Indies and eastern North America (Genbank numbers AY540195–AY540224). Details of polymerase chain reaction and sequencing methods were reported by Ricklefs and Fallon (2002) and Fallon et al. (2003). *Plasmodium falciparum* was used as the outgroup for rooting. A phylogenetic tree for the 69 parasite haplotypes was obtained using MrBayes 2.01 (Huelsenbeck, 2001) to fit a general time reversible model with sites partitioned by codon position. Simulations were run with four chains for 2,100,000 steps with a 100,000-step burn-in period and sampling every 100 steps. Based on the results of an initial search, 13 nodes in the phylogeny with high posterior support were constrained to increase potential support for other nodes, and the program was rerun to obtain a final phylogenetic tree. Another tree based on 21 cytochrome *b* sequences of 800 bp independently recovered all 13 constrained nodes with 99+% posterior credibility. In the final tree used in this study, 38 of 68 nodes had credibility values of >95%.

A phylogenetic tree for 44 of 50 host species (Appendix 1) was constructed from 802 bp of cytochrome *b* sequence obtained from GenBank and from original sequences, using the same settings in MrBayes as for the parasite sequences. Following an initial run, eight nodes having 35–84% credibility were constrained on the basis of evidence from DNA hybridization (Sibley and Ahlquist, 1990) and current avian taxonomy. In the constrained phylogeny, 13 of the remaining 35 nodes had credibility values of >95% and there were 9 more of >90%. Uncertainty in both the parasite and host trees inevitably adds noise to analyses of cospeciation and favors host switching in the reconciliation of the trees. However, if cospeciation were a prominent feature of the malaria–bird relationship, it should be possible to detect its signal in our analysis.

Host phylogenetic conservatism was assessed by a statistical test of association between parasite and host lineages based on the probability that a parasite or parasite clade is restricted to a single family of avian hosts. Taxonomy follows the American Ornithologists' Union (1998) checklist. The reconciliation analyses were based on phylogenies produced by earlier runs of MrBayes 2.01 using four chains with 530,000 steps and a burn-in period of 30,000 steps; these phylogenies were nearly identical to those produced by 2,100,000 step runs.

Results

Species Concepts

To study cospeciation, we must know what a species is. Species names for birds are widely accepted, and there is little controversy concerning the species of passerine birds included in our phylogenetic analysis. Species concepts for malaria parasites are another matter. Current Latin binomials for *Plasmodium* and *Haemoproteus* taxa are based on a small set of morphological characters, primarily the size, shape, and number of granules in parasites observed in blood smears (Coatneyi et al., 1971; Cogswell, 2000), and on the concept that malaria parasites are host specialists, at least to family (Bennett et al., 1993, 1994). Recent molecular work suggests that this approach is invalid and that a molecular phylogenetic definition of lineages is more appropriate. However, in the absence of additional genetic markers we cannot assess genetic diversity within interbreeding populations of parasites. Lacking population genetic information, one must adopt an arbitrary level of genetic divergence to distinguish clades of lineages as "species." Perkins (2000) suggested a cutoff of 3% sequence divergence in the cytochrome *b* gene

of malaria parasites of lizards for inclusion within a species. Because mitochondrial sequence divergence in malaria parasites may be substantially slower than it is in their vertebrate hosts (Ricklefs and Fallon, 2002), 3% may be too conservative. Named species of primate malaria parasites differ by as little as 1% sequence divergence (Escalante et al., 1998). The mitochondrial genomes of 100 *Plasmodium falciparum* parasites recovered from human populations worldwide revealed seven haplotypes differing by no more than two nucleotide substitutions in the cytochrome *b* gene and only two haplotypes differing by a single substitution within the 384-bp region used in this analysis (Joy et al., 2003). Fallon et al. (2003) grouped distinct clusters of closely related cytochrome *b* haplotypes averaging 0.6% genetic distance, or approximately two nucleotide substitutions, but differing from other clusters by ≥2% sequence divergence.

The only other character presently available to resolve the issue of parasite species limits is host distribution. We reasoned that closely related parasite haplotypes in the same area recovered consistently from different hosts probably do not represent interbreeding populations but rather independent populations differentiated genetically with respect to host suitability. Whether this criterion is valid or not, we have found it difficult to apply because our samples of parasites from a given region are too small for statistical assessment. Here, we report two anecdotal cases of parasite lineages differing by two nucleotides (about 0.6% sequence divergence) recovered from different avian hosts. Both examples are from North America. In the first case, one lineage of parasite was recovered from four northern parulas (*Parula americana*: Parulidae), and the other was from an eastern bluebird and a western bluebird (*Sialis sialia* and *S. mexicanus*: Turdidae). In the second case, one lineage was recovered from eight individuals representing six species of nine-primaried oscine passerine (Parulidae, Emberizidae, etc.) and the other was from three tufted titmice (*Baeolophus bicolor*: Paridae). Many other pairs of haplotypes differing by one or two nucleotides were recovered from different species but not in sufficient numbers to provide any statistical confidence. When more divergent haplotypes were recovered from the same host, we could not distinguish between a single panmictic population and different species occurring within a single host lineage. Accordingly, we arbitrarily decided to treat each haplotype as a separate entity in this analysis. If this practice caused errors, they would be on the side of excessive parasite speciation within host lineages (duplication).

Parasite Lineages

We obtained 525 cytochrome *b* sequences representing 69 distinct parasite mitochondrial DNA (mtDNA) haplotypes recovered from 50 passerine host species. Forty parasite haplotypes were observed only once, and eight additional haplotypes were each obtained from a single host species. At the other extreme, one West Indian parasite haplotype was represented by 163 sampled sequences, and one haplotype from Missouri was recovered from 16 host species.

The 50 host species were grouped into 10 distinct clades for the purpose of quantifying host sharing: Vireonidae + Corvidae, 5 species; Turdidae, 4; Mimidae, 7; Paridae, 2; Hirundinidae, 1; Troglodytidae, 1; Parulidae, 14; Icteridae, 3; Cardinalidae + *Piranga*, 6; and Emberizidae, 7. Among eight parasite haplotypes recovered from two hosts, four of the host pairs were in the same genus, three were in the same clade, and one spanned different clades. Of the five parasite haplotypes recovered from three hosts, all belonged to the same genus in two cases, to the same host clade in two cases, and to two different clades in one case.

The probability of drawing two hosts from the same one of these clades at random is

$$H = \frac{\sum_{i=1} n_i(n_i - 1)}{N(N-1)},$$

where n_i is the number of hosts in clade i and N is the total number of hosts (Ricklefs and Fallon, 2002). In this case, $H = 0.137$. Therefore, the probability of finding seven of eight pairs of hosts in the same clade is approximately $P = 10^{-5}$ (binomial distribution). Similarly, of 21 terminal pairs of sister parasite haplotypes, 10 were found exclusively in the same host clade ($P = 0.0002$). These results are robust to any rearrangement of species into clades defined by other criteria (e.g., Sibley and Ahlquist, 1990). Thus, the general host conservatism found by Ricklefs and Fallon (2002) also is evident in this larger sample of a more narrowly circumscribed—with respect to geography and host taxonomy—set of parasite lineages.

Parasite Phylogeny

A phylogenetic tree of parasite lineages is shown in Figure 1. The distribution of hosts on this tree is indicated by membership in three major clades of oscine passerines: the nine-primaried oscines, which include 30 species in the families Parulidae, Icteridae, Cardinalidae, and Emberizidae; 5 species in the related families Vireonidae (4 species) and Corvidae (1 species); and 15 species in the related muscicapoid and sylvioid families Turdidae, Mimidae, Paridae, Hirundinidae, and Troglodytidae. At this level of analysis, host clades appear to be clustered, as indicated by the significant test results. In addition, parasites recovered from vireonid–corvid hosts appear to be in more basal positions within the *Haemoproteus* clade than are parasites recovered from nine–primaried oscines, paralleling the more basal position of vireonids and corvids within the passeriform phylogeny (Sibley and Ahlquist, 1990; Barker et al., 2002). However, each of the host clades also appears in multiple locations within the parasite phylogeny (Bensch et al., 2000; Ricklefs and Fallon, 2002; Waldenström et al., 2002), which is indicative of host switching in the past.

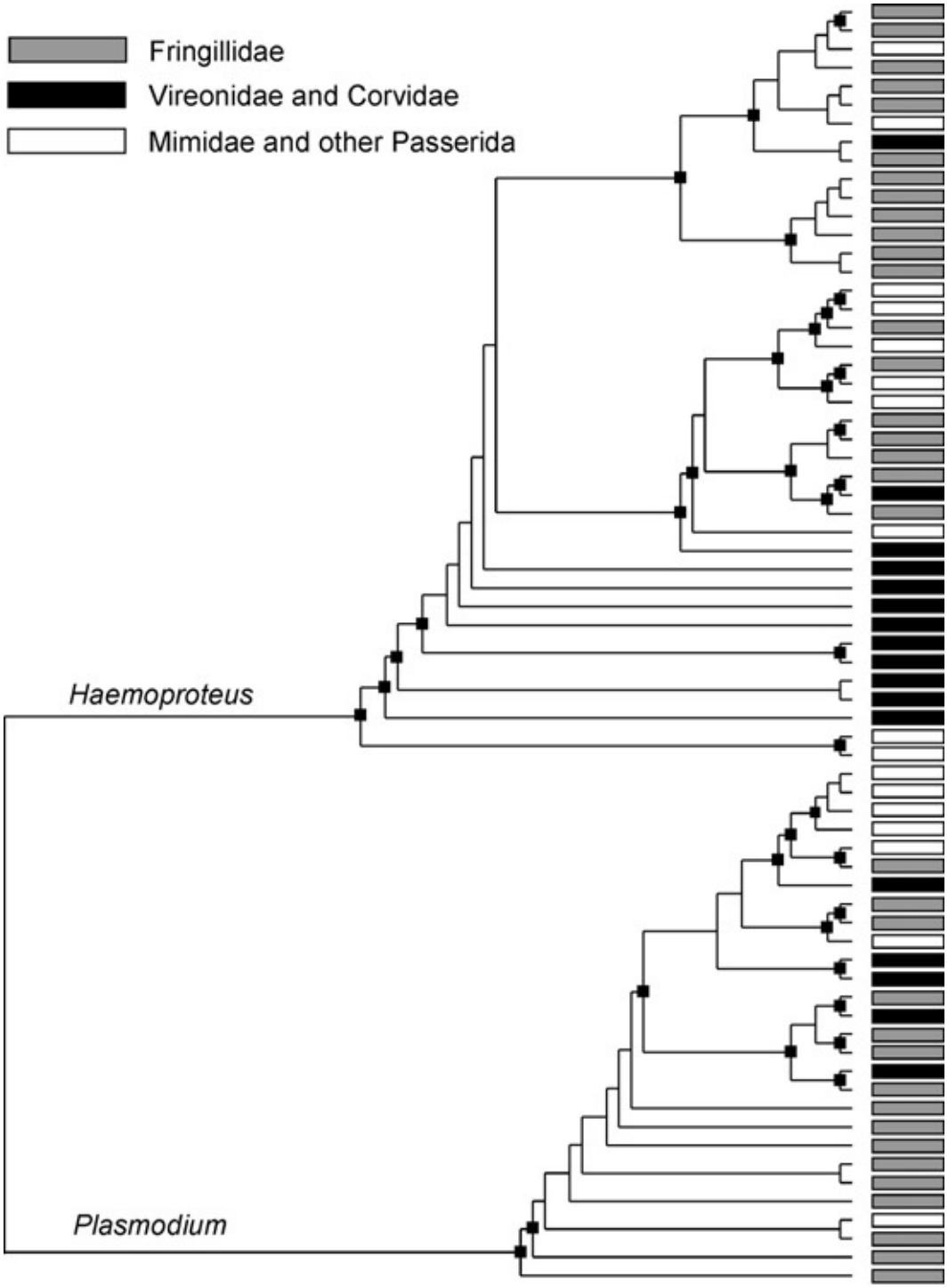

FIGURE 1. Phylogenetic tree based on a Bayesian analysis of 350 bp of mitochondrial cytochrome *b* sequence from 69 lineages of avian *Plasmodium* and *Haemoproteus* parasites. The human malaria parasite *P. falciparum* was used to root the phylogeny. Major taxonomic groupings of avian hosts are indicated by different rectangles. The Fringillidae includes the nine-primaried oscine families Parulidae, Emberizidae, Cardinalidae, and Thraupidae. Solid squares indicate nodes with >95% posterior probability.

Cospeciation

We applied several widely used approaches to assess the correspondence between parasite and host phylogenetic trees. These approaches were designed for systems having a high degree of cospeciation, and their efficacy appears to vary considerably (e.g., Dowling, 2002). The software program Component (Page, 1993) reconciles parasite and host trees by duplicating clades necessary to match the two trees according to the observed distribution of parasites on host taxa. Host switching is not incorporated in this program, although tree pruning (extinction) is. Host switching manifests itself in this program by duplication events followed by extinction. TreeMap2, a more recent program for tree reconciliation (Charleston, 1998; Page and Charleston, 1998), cannot handle phylogenies as large as those in this study. TreeFitter, written by Fredrik Ronquist (http://www.ebc.uu.se/systzoo/research/treefitter), provides an event-based comparison of trees and uses parsimony criteria to estimate the number of codivergence (cospeciation), duplication, sorting (partial extinction), and switching events required to match a parasite phylogenetic tree to that of the hosts (Ronquist, 1997). Both Component and TreeFitter assess estimates of event frequencies against results for randomized host trees. ParaFit (Legendre, 2001) provides a global test of host–parasite cospeciation. ParaFit incorporates a matrix approach to the similarity of parasite and host trees based on phylogenetic distance matrices for the parasites and the hosts and an incidence matrix describing the distribution of parasites across host lineages (Desdevises et al., 2002; Legendre et al., 2002). By comparing observed matrix values to those obtained from randomization of the incidence matrix, ParaFit provides statistics for cospeciation globally within the entire data set and for each observed parasite–host link.

Our analyses included 65 parasite lineages, 44 host species, and 121 host–parasite links. Component produced the following reconciled tree statistics: 52 duplications, resulting in 2,015 total leaves (1,950 leaves added), and 466 losses of clades. By comparison, a system obeying strict cospeciation produced no duplications or losses. The large number of duplications and subsequent losses required to reconcile the parasite tree to that of the host indicates that cospeciation is a relatively unimportant architect of the present distribution of parasites across host taxa. Thus, host switching permeates the parasite and host trees. However, some significant association of parasites on the host phylogenetic tree does occur, because more leaves were added to reconcile each of 100 randomized host trees (1,980–2,380) than were needed reconcile the observed tree (1,950).

TreeFitter compares the estimated number of events to the numbers obtained with randomized trees to test the correspondence between parasite and host phylogenies against a null model. One can assign costs to each type of event to weight its probability of occurring. The estimated numbers of each type of event depend on the cost assignments. Using the default costs of codivergence = 0, duplication = 0, sorting = 1, and switching = 2, the analysis found no significant signal for codivergence (cospeciation) in the overall data set (Table 1). Duplication of parasite lineages within hosts was significantly more frequent and switching was significantly less frequent than expected at random given the default cost structure. These results differ from those of the analysis of Ricklefs and Fallon (2002), who found significant cospeciation. The discrepancy undoubtedly results from the nonrandom sampling of host taxa and the more global nature of the earlier study, in which unique clades of hosts and their parasite lineages were restricted to different continents.

TABLE 1. Number of events (expressed as ranges that result in equal total costs) experienced by parasite lineages as required by TreeFitter to reconcile parasite and host phylogenetic trees under different cost structures.

Event costs[a]	Cost	Codivergence	Duplication	Sorting	Switching
0, 0, 1, 2	81	2–6	19–22[b]	1–3	39–40[c]
1, 1, 1, 1	64	0–12[b]	1–22[b]	0–0	41–63[c]
0, 1, 1, 1	52	12–16[b]	1–4	0–4	41–51[c]
1, 0, 1, 1	42	0–1	22–22[b]	0–0	41–42[c]
1, 1, 0, 1	64	0–31	1–64	0–505	0–63
1, 1, 1, 0	1	0–0	1–1	0–0	63–63

[a] Event costs are for codivergence (cospeciation), duplication (within-host speciation), sorting (extinction), and switching, respectively.

[b] The number of events significantly exceeds that for randomized trees ($P < 0.05$).

[c] The number of events is significantly less than that for randomized trees ($P < 0.05$).

We conducted separate analyses in TreeFitter with even cost structure and with the cost of each type of event individually downweighted in separate analyses (Table 1). The total event costs of the observed trees were always significantly less than those of randomized trees ($P < 0.05$), demonstrating correspondence between the host and parasite trees at some level. In these analyses, codivergence occurred significantly more frequently than in the randomized trees when costs were even and when codivergence was downweighted. The lowest total cost resulted from downweighting duplications, reflecting the frequent occurrence of related parasite lineages in the same hosts. For most cost structures, switching occurred less frequently than in randomized trees, again reflecting the general conservatism of parasite distribution. When the cost of switching was downweighted, tree reconciliation required 63 switching events and only a single duplication.

These results suggest that the parasite phylogeny is structured with respect to the host phylogeny, that switching is the most prominent type of event, and that both codivergence and especially duplication could have played important roles in the evolutionary diversification of the parasites. The relative proportions of the different events depend on their relative costs, which make any conclusions drawn from the analysis tentative.

In the application of ParaFit to our data, the global test of cospeciation had a value of 0.437 ($P = 0.63$), indicating no significant tendency for cospeciation in

this analysis. Of 121 parasite–host links, 7 (5.8%) had probabilities of <0.05 and 5 more (10%) had probabilities of <0.10, almost exactly what one would expect on the basis of random chance.

Discussion and Conclusions

Significant restriction of related parasite lineages to host clades provides strong support for conservatism in the distribution of malaria parasites across their avian host taxa. Event-based reconciliation of parasites to host trees by Component and TreeFitter also supported host conservatism and cospeciation under some event cost structures. Yet the global assay of cospeciation implemented by ParaFit lacked significance. This paradox probably is resolved by the high frequency of host switching, sometimes across great host taxonomic distances, relative to cospeciation. Host switching would obliterate evidence of codivergence at depth in the phylogenetic tree of the parasites, leaving information on codivergence only close to the tips of the phylogeny. Parasite species concepts, support for phylogenetic relationships, and sampling are sufficiently problematic at this point that even this shallow signal cannot be used to demonstrate cases of one-to-one cospeciation in parasites and their hosts.

This finding leads us to consider whether a parasite can show significant host conservatism in the absence of codivergence. How we define parasite species may have some bearing on this issue. An individual parasite lineage identified as a single cytochrome *b* haplotype has been recovered from up to 16 different host species, sometimes including more than one family of passerine bird. If these haplotypes belonged to a single panmictic parasite population, this would suggest that some parasite lineages are capable of infecting a wide variety of hosts and that host specificity should not be regarded as a signature attribute of malaria parasites. The infrequent recovery of a parasite from a distant host might represent a spillover infection from a primary host that could not be sustained in the absence of the reservoir in the typical host. In this case, extreme host breadth would result from accidental and transient events rather than incipient host sharing between taxonomically distant hosts.

Broadly distributed parasites might also represent clades of differentiated host specialists that cannot be distinguished by cytochrome *b* variation. Comparison of the host and parasite genetic distance matrices suggests that malaria cytochrome *b* evolves more slowly (Ricklefs and Fallon, 2002) in contrast to the usual situation in which parasite sequence divergence outpaces that of the host (Hafner et al., 1994; Page et al., 1998). If malaria parasites do evolve more slowly than their hosts, mtDNA sequence divergence may not provide sufficient resolution of evolutionary relationships.

Alternatively, speciation in malaria parasites may require substantial evolution before reproductive isolation is achieved, and many related haplotypes might simply makeup a single species. In this case, what we label as conservatism of host distribution among closely related lineages of parasite would be the result of host specialization by a single parasite population. Anecdotal evidence suggests that haematozoan clades with as little as 0.5% mtDNA sequence divergence might be specialized on different hosts, hinting at their being different species. However, separation of the haploid phases of the parasite life cycle in the vertebrate host does not preclude sexual reproduction and recombination of genotypes from different vertebrate host specialists in the dipteran vector. The nature of species in malaria parasites will only be resolved by analyzing multiple markers from independent parts of the parasite genome.

The patterns of association between malaria parasites and their hosts could be explained if host species acquired new parasites primarily by the switching of parasites from other hosts, but the propensity of parasites to share hosts was restricted primarily to relatively small taxonomic groups (Charleston and Robertson, 2002). This finding raises questions about the origin of new species within clades of parasites and the subsequent spread of parasite clades to new hosts. Parasite speciation might require geographical isolation, but the scale of the distance over which this isolation might take place is not well understood. Parasite lineages recovered by Ricklefs and Fallon (2002) from North America, Europe, and Africa largely differ, but there was also little overlap in the taxonomy of the hosts between the continents, so this is not a fair test of geographic structure in parasite lineages. Furthermore, parasite lineages from any one location were widely distributed throughout the parasite phylogeny, suggesting at least some mixing of malaria faunas between continents. We found several cases of parasite sharing, most likely through migrating birds, between hosts in eastern North America and the West Indies (see also Waldenström et al., 2002). This finding suggests that the scale of geographic isolation required for speciation would be quite large, perhaps greater than the range of most host species. Presumably, parasite lineages can move within the geographic distribution of a single host as rapidly as infected individuals disperse to new regions, providing that suitable vectors exist.

It is also possible that species formation occurs primarily following the acquisition of novel hosts, which is facilitated by mutations that allow parasites to circumvent the immune defenses of the new host. Such a switch would generally occur among closely related hosts, but more distant switches might establish conditions for strong divergent selection locally (Dieckmann and Doebeli, 1999; Doebeli and Dieckmann, 2000), leading to sympatric speciation by a model similar to that of host plant switching in tephritid flies and other groups (Bush, 1994; Abrahamson et al., 2001; Via, 2001; Berlocher and Feder, 2002). Such a model has been suggested for the monogenean parasites of fish by Zietara and Lumme (2002).

Significant duplication (speciation of parasites within host lineages) identified by TreeFitter under some event cost structures also poses a significant challenge. For example, of the 15 uppermost lineages of parasite in the tree illustrated in Figure 1, 6 were recovered from summer

tanagers (*Piranga rubra*); other hosts represented in this parasite clade included the scarlet tanager (*P. olivacea*), northern catbird (*Dumetella carolinensis*), hooded warbler (*Wilsonia citrina*), Kentucky warbler (*Oporornis formosus*), and red-eyed vireo (*Vireo olivaceus*), all from the same site in southern Missouri, and the Puerto Rican bullfinch (*Loxigilla portoricensis*) and pearly-eyed thrasher (*Margarops fuscatus*) from Puerto Rico. The presence of several related parasite lineages in a single host suggests duplication, as revealed by TreeFitter, but the tangle of other hosts harboring the same clade of parasites suggests that speciation events might have occurred between hosts followed by a broadening of host distribution involving further switching, often back to the original host.

The complex relationships discovered between malaria parasites and their avian hosts suggest that more extensive sampling of parasite lineages with several genetic markers will be productive. Multiple markers will help to establish the phylogenetic limits of interbreeding populations and provide a workable species concept for malaria parasites. Sympatric speciation between hosts would be implied by frequent occurrence of sister parasite "species" in the same region, but broader regional surveys would be required to examine the possibility of allopatric speciation of parasites within a single host species. Although event-based approaches to tree reconciliation are suggestive, direct identification of events through dense sampling of parasites and their hosts will be required to provide realistic estimates of the costs (probabilities) of different types of events. Regardless of the outcome of such studies, it is clear that the malaria–avian host system is extremely dynamic and complex.

ACKNOWLEDGMENTS

We are grateful to Steve Latta and Bethany Swanson for assistance in the field and laboratory. Field research in the West Indies would not have been possible without the support and encouragement of wildlife and conservation personnel in many governments and organizations of the Caribbean region. Blood sampling in the Missouri Ozarks was done in collaboration with the Missouri Forest Ecosystem Project (MOFEP), and we are grateful to John Faaborg and Paul Porneluzi. All fieldwork was conducted under animal care protocols approved by the University of Missouri–St. Louis IACUC. This research has been generously supported by the National Geographic Society, Smithsonian Institution, University of Missouri Research Board, and National Science Foundation. We thank Alex Scheuerlein, Kevin Johnson, James Whitfield, and an anonymous reviewer for insightful comments on the manuscript.

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Final acceptance 12 September 2003
Associate Editor: Kevin Johnson

APPENDIX 1. Host species used in the analyses of cospeciation and the source of cytochrome *b* sequence for reconstructing phylogenetic relationships. Taxonomy according to the American Ornithologists' Union (1998). Additional host species from which parasites were recovered but for which we lacked host cytochrome *b* sequence were Carolina wren (*Thryothorus ludovicianus*), eastern bluebird (*Sialia sialis*), prairie warbler (*Dendroica discolor*), ovenbird (*Seiurus olivaceus*), black-and-white warbler (*Mniotilta varia*), and hooded warbler (*Wilsonia citrina*).

Family	Common name	Genus	Species	GenBank number	Source
Cardinalidae	northern cardinal	*Cardinalis*	*cardinalis*	AF284071	Grapputo et al. (2001)
Cardinalidae	indigo bunting	*Passerina*	*cyanea*	AF447372	T. Yuri, and D. P. Mindell (unpubl.)
Cardinalidae	rose-breasted grosbeak	*Pheucticus*	*ludovicianus*	AF310058	A. Sato, H. Tichy, C. O'Huigun, et al. (unpubl.)
Coerebidae	bananaquit	*Coereba*	*flaveola*		E. Bermingham and I. J. Lovette (unpubl.)
Corvidae	common crow	*Corvus*	*brachyrhynchos*	AF171067	C. R. Lage and I. Kornfield (unpubl.)
Emberizidae	dark-eyed junco	*Junco*	*hyemalis*	U26199	Dodge et al. (1995)
Emberizidae	Lesser Antillean bullfinch	*Loxigilla*	*noctis*	AF310041	A. Sato, H. Tichy, C. O'Huigun, et al. (unpubl.)
Emberizidae	Puerto Rican bullfinch	*Loxigilla*	*portoricensis*	AF489886	Burns et al. (2002)
Emberizidae	swamp sparrow	*Melospiza*	*georgiana*	U40184	R. M. Zink and R. C. Blackwell (unpubl.)
Emberizidae	eastern towhee	*Pipilo*	*erythrophthalmus*	AF284075	A. Grapputo, A. Pilastro, A. J. Baker, and G. Marin (unpubl.)
Emberizidae	black-faced grassquit	*Tiaris*	*bicolor*	AF310044	A. Sato, H. Tichy, C. O'Huigun, et al. (unpubl.)
Emberizidae	white-crowned sparrow	*Zonotrichia*	*leucophrys*	AF305764	J. D. Weckstein, R. M. Zink, R. C. Blackwell-Rago, et al. (unpubl.)
Hirundinidae	violet-green swallow	*Tachycineta*	*thalassina*	AY052449	L. A. Whittingham, B. Slikas, D. W. Winkler, and F. H. Sheldon (unpubl.)
Icteridae	red-winged blackbird	*Agelaius*	*phoeniceus*	AF290173	Klicka et al. (2000)
Icteridae	common grackle	*Quiscalus*	*quiscula*	AF089058	S. M. Lanyon, and K. E. Omland (unpubl.)
Mimidae	trembler	*Cinclocerthia*	*ruficauda*		E. Bermingham and I. J. Lovette (unpubl.)
Mimidae	gray catbird	*Dumetella*	*carolinensis*	AF151395	E. Pasquet, A. Cibois, F. Baillon, and C. Erard (unpubl.)
Mimidae	pearly-eyed thrasher	*Margarops*	*fuscatus*		E. Bermingham and I. J. Lovette (unpubl.)
Mimidae	scaly-breasted thrasher	*Margarops*	*fuscus*		E. Bermingham and I. J. Lovette (unpubl.)
Mimidae	northern mockingbird	*Mimus*	*polyglottos*		E. Bermingham and I. J. Lovette (unpubl.)
Mimidae	brown thrasher	*Toxostoma*	*rufum*		E. Bermingham and I. J. Lovette (unpubl.)
Paridae	tufted titmouse	*Baeolophus*	*bicolor*	D38314	Chikuni et al. (1995)
Paridae	black-capped chickadee	*Parus*	*atricapillus*	U60770	L. Kvist, M. Ruokonen, M. Orell, and J. Lumme (unpubl.)
Parulidae	myrtle warbler	*Dendroica*	*coronata*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	yellow-throated warbler	*Dendroica*	*dominica*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	magnolia warbler	*Dendroica*	*magnolia*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	chestnut-sided warbler	*Dendroica*	*pensylvanica*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	blackpoll warbler	*Dendroica*	*striata*	AF290176	Klicka et al. (2000)
Parulidae	common yellowthroat	*Geothlypis*	*trichas*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	worm-eating warbler	*Helmitheros*	*vermivorus*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	yellow-breasted chat	*Icteria*	*virens*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	Kentucky warbler	*Oporornis*	*formosus*		E. Bermingham and I. J. Lovette (unpubl.)
Parulidae	northern parula	*Parula*	*americana*	AF256502	Lovette and Bermingham (1999)
Parulidae	northern waterthrush	*Seiurus*	*novaboracensis*		E. Bermingham and I. J. Lovette (unpubl.)
Sturnidae	European starling	*Sturnus*	*vulgaris*	AF378103	H. F. James, P. G. P. Ericson, B. Slikas, et al. (unpubl.)
Thraupidae	scarlet tanager	*Piranga*	*olivacea*	AF011775	Burns (1998)
Thraupidae	summer tanager	*Piranga*	*rubra*	AF011779	Burns (1998)
Turdidae	wood thrush	*Hylocichla*	*mustelina*	AY049504	K. Winker and C. L. Pruett (unpubl.)
Turdidae	western bluebird	*Sialia*	*mexicanus*		S. M. Fallon (unpubl.)
Turdidae	American robin	*Turdus*	*migratorius*	AF197835	Cracraft and Feinstein (2000)
Vireonidae	black-whiskered vireo	*Vireo*	*altiloquus*	U12305	Murray et al. (1994)
Vireonidae	white-eyed vireo	*Vireo*	*griseus*	U12294	Murray et al. (1994)
Vireonidae	red-eyed vireo	*Vireo*	*olivaceus*	X74260	Helm-Bychowski and Cracraft (1993)
Vireonidae	blue-headed vireo	*Vireo*	*solitarius*	AY030137	C. Cicero and N. K. Johnson (unpubl.)