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A SINGLE ANCIENT ORIGIN OF BROOD PARASITISM IN AFRICAN FINCHES: IMPLICATIONS FOR HOST-PARASITE COEVOLUTION

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Abstract.—Robust phylogenies for brood-parasitic birds, their hosts, and nearest nesting relatives provide the framework to address historical questions about host-parasite coevolution and the origins of parasitic behavior. We tested phylogenetic hypotheses for the two genera of African brood-parasitic finches, *Anomalospiza* and *Vidua*, using mitochondrial DNA sequence data from 43 passeriform species. Our analyses strongly support a sister relationship between *Vidua* and *Anomalospiza*, leading to the conclusion that obligate brood parasitism evolved only once in African finches rather than twice, as has been the conventional view. In addition, the parasitic finches (Viduidae) are not recently derived from either weavers (Ploceidae) or grassfinches (Estrildidae), but represent a third distinct lineage. Among these three groups, the parasitic finches and estrildids, which includes the hosts of all 19 *Vidua* species, are sister taxa in all analyses of our full dataset. Many characters shared by *Vidua* and estrildids, including elaborate mouth markings in nestlings, unusual begging behavior, and immaculate white eggs, can therefore be attributed to common ancestry rather than convergent evolution. The host-specificity of mouth mimicry in *Vidua* species, however, is clearly the product of subsequent host-parasite coevolution. The lineage leading to *Anomalospiza* switched to parasitizing more distantly related Old World warblers (Sylviidae) and subsequently lost these characteristics. Substantial sequence divergence between *Vidua* and *Anomalospiza* indicates that the origin of parasitic behavior in this clade is ancient (~20 million years ago), a striking contrast to the recent radiation of extant *Vidua*. We suggest that the parasitic finch lineage has experienced repeated cycles of host colonization, speciation, and extinction through their long history as brood parasites and that extant *Vidua* species represent only the latest iterations of this process. This dynamic process may account for a significantly faster rate of DNA sequence evolution in parasitic finches as compared to estrildids and other passerines. Our study reduces by one the tally of avian lineages in which obligate brood parasitism has evolved and suggests an origin of parasitism that involved relatively closely related species likely to accept and provide appropriate care to parasitic young. Given the ancient origin of parasitism in African finches, ancestral estrildids must have been parasitized well before the diversification of extant *Vidua*, suggesting a long history of coevolution between these lineages preceding more recent interactions between specific hosts and parasites.

Key words.—Base composition, brood parasitism, mitochondrial DNA, optimization alignment, Passeriformes, phylogeny, *Vidua*.

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Some of the clearest examples of coevolution are provided by avian brood parasites and their hosts (Rothstein 1990; Payne 1997a). Adaptations of parasitic species to secure parental care from hosts and counter-adaptations of hosts to avoid or reduce the negative effects of parasitism have been studied extensively in selected species (e.g., Payne 1977; Davies and Brooke 1988). These studies have considered a variety of functional, mechanistic, and developmental aspects of host-parasite interactions (e.g., Rothstein 1982; Lotem et al. 1995; Kilner et al. 1999), but questions about the evolutionary history of brood parasitism have received less attention.

Well-corroborated phylogenies for brood parasitic species, their hosts, and their closest nonparasitic relatives provide the basis for addressing a variety of questions about both the origins of parasitic behavior and host-parasite coevolution. For example, Payne (1977, 1998a) suggested three possible precursors to obligate parasitism: (1) facultative parasitism of conspecifics and/or other species; (2) cooperative nesting; and (3) the use or takeover of nests built by other species. The presence or absence of these behaviors in the nesting relatives of brood parasitic birds provides a test of these alternative hypotheses for the evolution of parasitism. Additional questions that can be addressed using a phylogeny include: How many times has obligate brood parasitism evolved? Are behavioral and morphological characters shared by parasites and their hosts due to common ancestry or selection on parasites to mimic their hosts? What are the relative ages of different parasitic lineages? Researchers have begun to address some of these historical questions for different groups of parasitic birds (e.g., Lanyon 1992; Hughes 1996, 2000; Klein and Payne 1998; Aragon et al. 1999; Gibbs et al. 2000).

Molecular phylogenies for the parasitic cowbirds (*Molothrus* spp.) and other icterids (Lanyon 1992; Johnson and Lanyon 1999; Omland et al. 1999) confirm a single origin of brood parasitism in this group but also show that the bay-winged cowbird (''*M.*'' *badius*), a parental species that takes over other species' nests, is not closely related to the parasitic cowbirds. Thus, the phylogeny provides no support for nest takeover as a precursor to obligate brood parasitism in cowbirds. In addition, the nearly identical appearance of young *M. badius* and *M. rufoaxillaris*, the specialist brood parasite of *M. badius* (Hudson 1874; Lichtenstein 2001), is clearly the result of evolved mimicry rather than common ancestry (Lanyon 1992). Klein and Payne (1998) compared phylogenies of brood parasitic finches (*Vidua* spp.) with those of their estrildid hosts to test the hypothesis that these host-

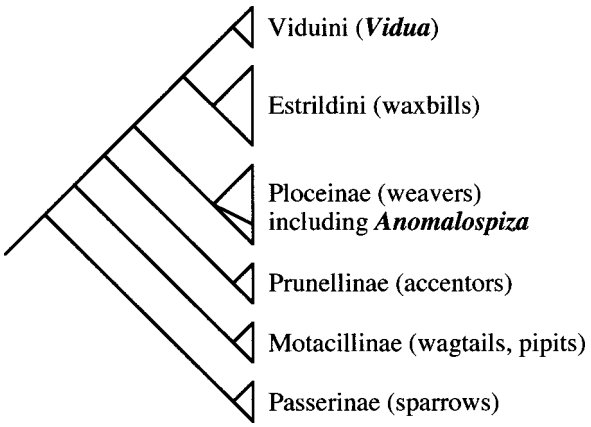


FIG. 1. Phylogenetic hypothesis for Old World finches based on Sibley and Ahlquist (1990) and Sibley and Monroe (1990). Estrildini and Viduini were treated as tribes within Estrildinae (Sibley and Monroe 1990). This phylogeny implies independent origins of obligate brood parasitism in *Vidua* and *Anomalospiza*. In this paper, we treat most of the clades in this tree as family-level taxa (see Table 1).

specific parasites evolved in a process of host-parasite cospeciation. Their analysis indicated a very recent origin of the parasitic species, supporting a model of parasite speciation through host colonization.

Current views on avian relationships suggest seven independent origins of obligate brood parasitism among birds (Sibley and Monroe 1990; Johnsgard 1997). This includes three origins within Passeriformes: once in the New World cowbirds (Icterinae) and twice in African finches, represented by the genera *Vidua* and *Anomalospiza* (Payne 1996, 1997a,b, 1998a). The *Vidua* finches, including 10 indigobird species and nine long-tailed whydahs, are of particular interest because of their high degree of host specificity and relatively benign effects on hosts as compared to other parasitic birds. All *Vidua* parasitize estrildid finches (family Estrildidae) and, with a few exceptions, each is exclusively associated with a different host species. Adult males of most *Vidua* species mimic the songs of their respective hosts, and nestlings mimic the mouth markings and begging behavior of host young. In contrast, the cuckoo-finch (*Anomalospiza imberbis*) parasitizes several species of Old World grass warblers (*Cisticola* spp. and *Prinia* spp.). Host young usually do not survive in nests that fledge a cuckoo-finch, and cuckoo-finch nestlings do not mimic host mouth markings or begging behavior (Friedmann 1960; Vernon 1964).

Although various phylogenetic hypotheses have been proposed for the two genera of parasitic finches, recently authors have placed *Vidua* with the estrildid finches, whereas *Anomalospiza* has been recognized as a weaver (family Ploceidae; e.g., Friedmann 1960). A phylogenetic hypothesis for the Old World finches based on DNA-DNA hybridization data (Sibley and Ahlquist 1990) is shown in Figure 1; although no molecular data were collected for *Anomalospiza*, it was included with ploceids in the classification of Sibley and Monroe (1990).

Behavioral and morphological characters also have been used to suggest alternative placements of the parasitic finches with respect to estrildids and ploceids, although such data have rarely been examined in a phylogenetic context. Chapin (1917, 1954), Friedmann (1960), and Kunkel (1969) reasoned that similar mouth markings in young *Vidua* and estrildids were evidence of their common ancestry. Other synapomorphies of *Vidua* and estrildids include the absence of three muscles present in other Old World finches (Bentz 1979) and several plumage characters (Morlion 1980). Neunzig (1929), in contrast, argued for a relationship between *Vidua* and ploceids, reasoning that similarities in egg color, mouth markings, and juvenile plumage between *Vidua* and their estrildid hosts were due to evolved mimicry rather than shared ancestry. Nicolai (1964) described *Vidua* courtship displays and calls as being derived from those of ploceids, whereas Ziswiler (1965, 1967) concluded that *Vidua* is more similar to the euplectines (Ploceidae) than to estrildids based on structures of the horny palate and digestive tract. Small outer primaries (and long tails) in both *Vidua* and widowbirds (*Euplectes* spp.) have led various authors to group them together, either within Ploceidae (Delacour and Edmund-Blanc 1933–1934) or along with estrildids in Viduinae (as a subfamily within Ploceidae, Sharpe 1890).

By virtue of its yellow plumage, *Anomalospiza* was originally described as a cardueline canary and included in Fringillidae (e.g., Sharpe 1888). Chapin (1917, p. 259) grouped *Anomalospiza* with the estrildid finches, citing bill shape and the small outer primary, whereas Shelley (1905) included both *Vidua* and *Anomalospiza* (along with estrildids and euplectines) in Viduidae. *Anomalospiza* also shares with *Vidua* a common distribution in Africa, juvenile plumage that is distinct from the adult female's, and an unpneumatized frontal area in the skull of adults (Chapin 1954; Friedmann 1960; Williams and Keith 1962), a trait found in estrildids that breed as early as three months of age (Morel 1973), but in few adult ploceids (Disney 1980). Both genera are also obligate brood parasites, although brood parasitism was not recognized in *Anomalospiza* until 1917 (Roberts 1917). Citing these similarities, only Bannerman (1949) explicitly grouped the two parasitic genera in Viduinae. Following Chapin (1954), however, Friedmann (1960) treated *Anomalospiza* as a ploceid, concluding that it ''is clearly not closely related to the Viduinae.''

We collected mitochondrial DNA (mtDNA) sequence data from a broad sample of Old World songbirds to test alternative phylogenetic hypotheses for the two parasitic finch genera and their nesting relatives. Our objectives in this analysis were: (1) to test the hypothesis that *Vidua* and *Anomalospiza* are sister taxa, and therefore that obligate brood parasitism evolved only once in African finches; (2) to identify the sister group of the parasitic finches, providing a test of alternative hypotheses for the origins of parasitic behavior and insight into the evolution/coevolution of behavioral and morphological traits shared by brood parasites and their hosts; and (3) to estimate the age of the parasitic finch lineage, providing a measure of the historical duration of coevolutionary interactions between parasitic finches and their hosts.

Because of the relatively deep divergences among the taxa in our study, DNA sequence alignment and base composition bias were important issues in our phylogenetic analysis. Therefore, we also consider in some detail the use of optimization alignment (Wheeler 1996) and the contribution of

gap characters (representing nucleotide insertions and deletions) to inferences about the relationships among Old World finch families.

Materials and Methods

Taxa and DNA Sequencing

Taxa included in our analyses are listed in Table 1. We included individual *Anomalospiza* from Nigeria and Zambia and representatives of the four main clades within *Vidua*. We sampled a number of estrildids and ploceids, as well as representatives of each clade that Sibley and Ahlquist (1990) found to be most closely associated with these groups. These include Old World sparrows (Passeridae), pipits and larks (Motacillidae), accentors (Prunellidae), northern finches (Fringillidae), and New World nine-primaried oscines (Emberizidae), a group that includes the brood-parasitic cowbirds. The above groups were treated as subfamilies by Sibley and Monroe (1990) and were included in their Passeridae or Fringillidae. We also sampled representatives of six other oscine families to provide a broad test of the monophyly of the above taxa and of possible alternative placements for the parasitic finches. Representatives of the Laniidae and Monarchidae are included as an outgroup. Sibley and Monroe (1990) included these taxa in Corvida, the sister group to their Passerida. In general, we focus on relationships among finch families, leaving species-level relationships within families for future analyses with larger taxonomic samples.

Genomic DNA was isolated from 25 mg of muscle tissue or the calmus of a single inner primary or tail feather using a QIAamp Tissue Kit (Qiagen, Valencia, CA). For feathers, we added 30 μl of 100 mg/ml dithiothreitol (DTT) to the tissue digestion buffer (Cooper 1994). Feathers of *Eurocephalus*, *Terpsiphone*, and *Anomalospiza* were obtained from museum specimens ranging from 20 to 28 years old.

For all of the sampled taxa, we sequenced the entire mitochondrial genes for NADH dehydrogenase subunit 2 (ND2) and the small subunit ribosomal RNA (12S) along with portions of the transfer RNAs flanking each gene. Each gene was amplified and sequenced in two overlapping fragments. ND2 was amplified and sequenced with primers L5216 (or L5219), H5766, L5758, and H6313. (L and H numbers refer to the strand and position of the 3′ base in the published chicken sequence, Desjardins and Morais 1990.) The 12S gene was amplified and sequenced with primers L1267, H1858, L1753, and H2294.

A third mitochondrial region comprising almost all of the ND6 gene, tRNA-glutamine (tGlu), and the 5′ half of the control region (CR) was sequenced for 30 ingroup taxa (Passeridae and following in Table 1). Primers for this region worked poorly or not at all for the other 14 taxa. Primer L16225 (or L16150 or L16206) was paired with primer Finch5PR (5′-GCTTTTGGTGGAGTGCCATAG-3′) or Finch5PR2 (5′-CATTTCAGTRAMTGTCTGATGG-GGC-3′) to amplify ND6 and tGlu. Primers IndigoC1F1 (5′-TCTTCATGCTTTACAGGGTATG-3′), FireC1F1 (5′-TTTTCCTHNTGACTTTTAGGGTATG-3′), or Passer-C1F1 (5′-TCTATACTTTCAGGGTATGT-3′; C. Tarr, pers. comm. 1995) were paired with FinchC1R1 (5′-GGT-ATGGTCCTGAAGTTACAAC-3′) to amplify and sequence approximately the first 550 base pairs of the control region. Given the lower quality of DNA extracts from museum material, the ND6 region in *Anomalospiza* was amplified in two smaller pieces using primers ANOM.H16632 (5′-GCAGTTKCMTCCAACCCBTCTCC-3′) and Indigo.L16623 (5′-ACAACCARCCCHACCACCC-3′) in addition to L16225 and Finch5PR. Primer sequences not provided above can be found in Sorenson et al. (1999).

Our choice of loci (including protein-coding genes on both the light and heavy stands of the mtDNA, a ribosomal RNA gene, and the noncoding CR) was intended to provide phylogenetically informative molecular characters evolving under different functional constraints and at different rates. We reason that this approach maximizes the potential independence of the mtDNA characters in our analysis by reducing the potential for systematic biases (for an explanation of systematic bias, see Swofford et al. 1996).

PCR amplifications were in 50 μl total volume with 1.25 units AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA), 2.5 mM $MgCl_2$, 0.25 mM each dNTP, and 1 μM each primer. For extracts from museum skin, we used AmpliTaq Gold (Applied Biosystems) and 50 polymerase chain reaction (PCR) cycles to increase product yield. PCR products were gel-purified in 1.5% low-melt agarose, excised from the gel, and purified with a Gel Extraction Kit (Qiagen). Double stranded PCR products were sequenced directly using Taq DNA Polymerase FS (Applied Biosystems). Sequencing reaction products were run on an Applied Biosystems 377 DNA sequencer. Electropherograms were compared and reconciled using Sequence Navigator (Applied Biosystems; see Sorenson et al. 1999).

Requisite precautions, including the use of PCR primers with degenerate sites and the use of muscle or feather tissue but not blood as sources of DNA, were taken to avoid the unintended amplification of nuclear pseudogenes of mitochondrial origin (Sorenson and Fleischer 1996; Sorenson and Quinn 1998). In one *Anomalospiza* sample, there were two positions in ND2 with double-peaks, suggesting the coamplification of two slightly different sequences, but no other evidence of possible nuclear copies was observed. These two positions were coded as R (either adenine or guanine) in our data matrix.

Sequence data collected in this study have been deposited in GenBank (accession numbers AF407019–AF407133 and AF090341).

Phylogenetic Analyses

Phylogenetic analyses were conducted in PAUP*, version 4.0b4 (Swofford 2000) and POY (Gladstein and Wheeler 1996). Our dataset includes 3155–3168 base pairs per taxon for 30 ingroup taxa and 2098–2105 base pairs per taxon for 14 outgroup taxa. For analyses of the full dataset, the ND6/t-Glu/CR region was coded as missing for the outgroup taxa. Two segments of the CR comprising 18–66 nucleotides per taxon were excluded from all analyses because large insertions and deletions have clearly occurred in these regions, making contiguous gap characters nonindependent. We refer to other regions that were variable in length among taxa and for which there was no single unambiguous alignment as

TABLE 1. List of taxa for which DNA sequence data were collected. Taxa are listed following the classification of Campbell and Lack (1985), except that *Anomalospiza* is included in Viduidae on the basis of results presented here. Obligate brood parasites are denoted by an asterisk. DNA extracts obtained from feather are indicated by (f).

Taxon	Locality	Museum no.[1]	Tissue no.	
Laniidae				
Corvinella corvina	Gambia	UMMZ A857	A857	(f)
Eurocephalus anguitimens	Zimbabwe	UMMZ 202,542		(f)
Lanius senator	Gambia	UMMZ A525	A525	(f)
Monarchidae				
Elminia longicauda	Cameroon	UMMZ 232,419	A241	
Terpsiphone viridis	Swaziland	UMMZ 215,126		(f)
Sturnidae				
Sturnus vulgaris	Michigan	UMMZ 233,191	T701	
Pycnonotidae				
Pycnonotus barbatus	Cameroon	UMMZ 232,528	A144	
Sylviidae				
Camaroptera brevicaudata	Gambia	UMMZ A339	A339	(f)
Cisticola fulvicapilla	Zimbabwe	BWYO	A761	
Hypergerus atriceps	Gambia	UMMZ A345	A345	(f)
Locustella ochotensis	Japan	UMMZ 234,839	T1146	
Parisoma subcaeruleum	Zimbabwe	BWYO	A759	
Phylloscopus trochilus	Gambia	UMMZ A832	A832	
Alaudidae				
Eremophila alpestris	Arizona	UMMZ 227,635	T386	
Passeridae				
Passer domesticus	Michigan	UMMZ 228,398	T553	
Petronia dentata	Cameroon	UMMZ 232,531	A240	
Motacillidae				
Motacilla alba	Japan	UMMZ 234,748	T1194	
Prunellidae				
Prunella modularis	Great Britain	UMMZ A374	AF374	(f)
Prunella montanella	Russia	UWBM 44,004	CSW4550	
Ploceidae				
Ambylospiza albifrons	South Africa	UWBM 52,933	SAR6756	
Bubalornis albirostris	Gambia	UMMZ 234,183	A415	
Euplectes macrourus	Cameroon	UMMZ 232,429	A135	
Plocepasser mahali	Zimbabwe	UWBM 57,040	S23	
Ploceus ocularis	Malawi	NMM	A59	
Quelea quelea	Cameroon	UMMZ 232,530	A168	
Sporopipes frontalis	Nigeria	UMMZ 233,830	A287	
Estrildidae				
Amandava subflava	Cameroon	UMMZ 232,471	A208	
Chloebia gouldiae	(captive)	UMMZ 233,785	T807	
Estrilda astrild	Malawi	NMM	A26	
Hypargos niveoguttatus	Zimbabwe	BWYO	A24	
Lagonosticta sanguinodorsalis	Nigeria	UMMZ 233,840	A313	
Ortygospiza atricollis	Cameroon	UMMZ 232,472	A157	
Spermestes cucullatus	Cameroon	UMMZ 232,476	A137	
Viduidae				
*Vidua chalybeata**	Cameroon	UMMZ 232,516	A189	
*Vidua hypocherina**	(captive)	UMMZ 231,669	90.o31	
*Vidua macroura**	Malawi	UMMZ 231,387	A089	
*Vidua paradisaea**	Malawi	NMM	A081	
*Anomalopsiza i. imberbis**	Zambia	UMMZ 219,690		(f)
*Anomolospiza i. butleri**	Nigeria	UMMZ 216,978		(f)
Fringillidae				
Fringilla coelebs	Russia	UWBM 49,391	BKS1790	
Carduelis pinus	Michigan	UMMZ 227,858	T540	
Emberizidae				
Junco hyemalis	Alaska	UMMZ 234,014	T797	
Icteridae				
*Molothrus bonariensis**	Peru	LSUMZ 113,963	B-5181	
*Scaphidura oryzivora**	Bolivia	LSUMZ 134,021	B-9686	

[1] LSUMZ, Louisiana State University Museum of Zoology; NMM, National Museum of Malawi; BWYO, National Museum of Zimbabwe in Bulawayo; UMMZ, University of Michigan Museum of Zoology; UWBM, University of Washington Burke Museum.

''gap regions.'' Gap regions represent a total of 366 alignment positions in our final conventional alignment (see below) and include 260–274 nucleotides and 92–106 gap characters per taxon for the 30 ingroup taxa. The remaining, well-aligned portions of the dataset comprise 2910 alignment positions, including 22 positions with gap characters in one or more taxa. Gaps were treated as a fifth character state in parsimony analyses.

To explore differences in the phylogenetic signal contained in transitions and transversions, we completed a series of weighted parsimony analyses with increasingly severe down-weighting of transitions. For each set of parameters and/or taxa, we completed 100 replicate heuristic searches with random addition of taxa and tree-bisection-reconnection (TBR) branch-swapping. To quantify the support for individual clades, we determined bootstrap values (Felsenstein 1985) using full heuristic searches and 500 randomly resampled datasets and Bremer support indices (Bremer 1988) using the program TreeRot (Sorenson 1999).

Because sequences from 12S, the CR, and transfer-RNAs vary in length among taxa, sequence alignment was an important issue in the analysis of our data. Although gap regions are typically excluded from phylogenetic analyses, there is clear evidence that such regions contain useful phylogenetic information (Giribet and Wheeler 1999; see below). The challenge is to include this information in an unbiased and logically consistent manner. Optimization alignment (Wheeler 1996), as implemented in the program POY (Gladstein and Wheeler 1996), meets these requirements. Because tree searches and sequence alignment are combined in a single process, there is no opportunity for preconceived biases about relationships to influence the alignment and, in turn, the tree topology. Optimization alignment is also logically consistent in that the same criterion, minimizing the number of steps on a phylogenetic tree, is used for choice of both alignment and phylogenetic hypothesis. As a result, POY yields far more parsimonious alignments as measured by final tree length than the usual two step process of aligning sequences using an algorithm such as Clustal W (Thompson et al. 1994) and then using a different optimality criterion to select the best tree.

POY has several disadvantages, however. The use of heuristics to estimate tree length results in a slight, but unknown overestimation of the actual length of each tree (Wheeler 1998; and see below). Different scores are given to the same topology depending on how it is rooted (Wheeler 1996), an artifact of the heuristics used to estimate tree length (W. C. Wheeler, pers. comm. 1998). The program has no provision for counting multiple-base indels as single events, although this could be implemented within the optimization alignment framework (Wheeler 1998). While also advocating full use of the potential information present in gap regions, Simmons and Ochoterena (2000) describe a different method for coding gaps that attempts to solve the problem of nonindependence of contiguous gap characters. Their method, however, starts with aligned sequences and does not specify how variable-length sequences should be aligned in the first place. Especially for sequences in which most indel events appear to involve single nucleotides and where it is not possible, a priori, to construct a single unambiguous alignment of the data, we think optimization alignment as implemented by POY is the best solution to the joint problem of sequence alignment and choice of optimal topology.

Because analysis of our full dataset in POY produced two very different trees with similar scores (see Results), we explored the extent to which POY overestimated tree length using the following procedures. First, we delineated 25 separate gap regions in our dataset, each of which included short sequences of uncertain alignment. Then, using POY, we estimated the number of steps for each of these regions for all possible rootings of the six best trees found by POY. We evaluated each well-delineated gap region separately because a particular rooting of the tree might result in the best possible score for one gap region but an overestimate of the score for another region. We summed the smallest values obtained for each gap region plus the length of the well-aligned portions of the data set to obtain a corrected score for each tree. (We are not aware of similar procedures being used in other studies.) This lower corrected score, however, was still an overestimate of the minimum possible number of steps for that particular tree. We reached this conclusion in the following manner. For trees with the lowest corrected scores, we incorporated into our dataset the best implied alignment (a conventional static alignment output by POY) for each gap region and then improved it by trial and error, iteratively making small adjustments and determining whether each reduced the length of the tree, always adhering to the criterion that adjustments not increase tree length. We obtained a conventional static alignment with a score still smaller than any of the corrected scores from POY. We used this optimized, conventional alignment for subsequent analyses such as the determination of Bremer support indices and comparison of tree lengths for alternative phylogenetic hypotheses. We emphasize that by optimizing the alignment on one tree, node support metrics and differences in tree length between this tree and alternative topologies are maximized. It should also be noted that there are likely to be alternative, equally parsimonious optimizations of the alignment for a given tree topology and that the choice of a particular static alignment for subsequent analysis is somewhat arbitrary. However, minor differences between equally parsimonious alignments probably have minimal effect: most of the a posteriori adjustments we made to the alignment reduced the length of all trees with similar topologies.

We completed the above procedures only for the equal-weights analysis of the full dataset. For all other analyses, we report the original tree scores output by POY. To explore the sensitivity of the results to different transformation costs (e.g., Wheeler 1995), we completed analyses in POY both with equal weights for all changes and with transitions down-weighted by 50% or 80% relative to transversions and gaps. For each set of parameters and/or taxa, we completed 100 replicate searches with random addition of taxa and used the following options: noquick, slop = 1, checkslop = 5, maxtrees = 5.

Maximum-likelihood (ML) analyses were based on well-aligned portions of the dataset only, excluding 22 additional positions with a gap character in one or more taxa. We used MODELTEST (Posada and Crandall 1998) to select the model of sequence evolution that best fit our data. Likelihood

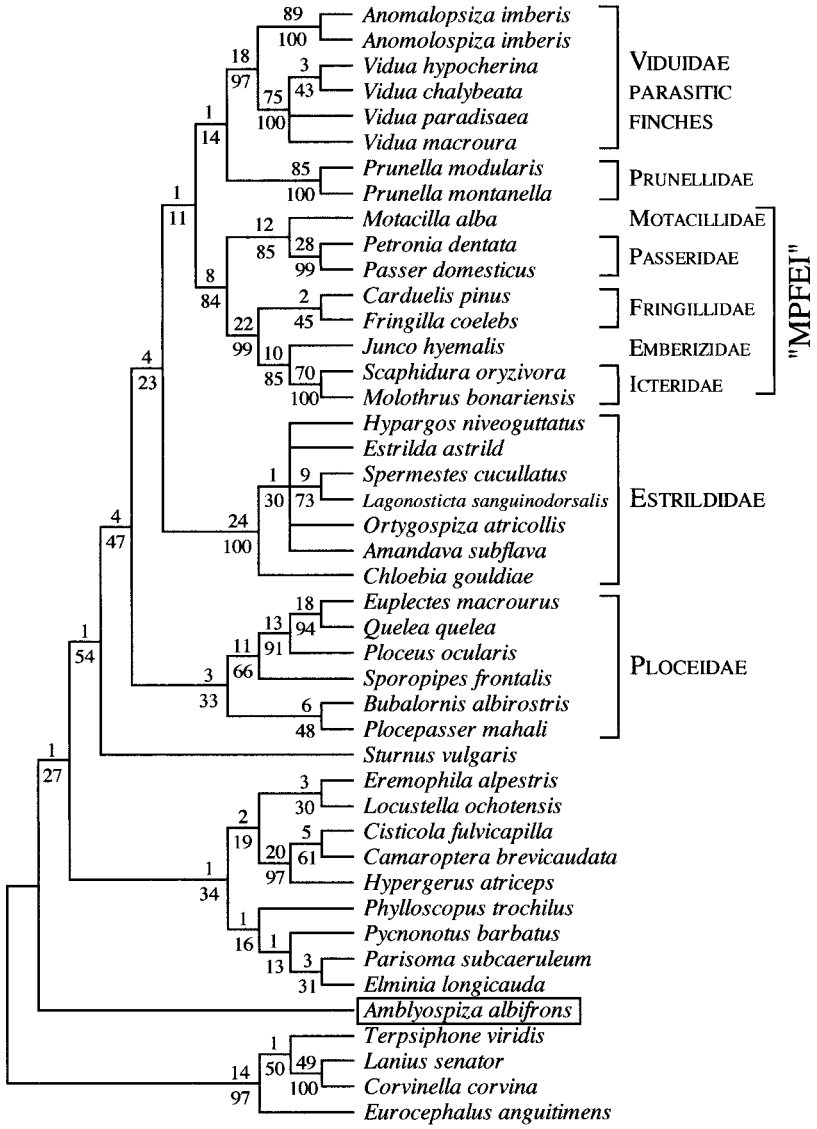


FIG. 2. Strict consensus of six most parsimonious trees of length 7773 (CI = 0.28, RI = 0.39) for well-aligned regions of the dataset only (2910 alignment positions). Bremer support indices and bootstrap values are shown above and below each node, respectively.

scores and parameters for various models were estimated in PAUP* for the best parsimony tree resulting from an optimization alignment analysis in POY with 50% down-weighting of transitions. A series of hierarchical likelihood-ratio tests was then conducted in MODELTEST to determine if increasingly complex models resulted in significant improvements in likelihood scores. The general time reversible (GTR) model of nucleotide substitution, with unequal nucleotide frequencies, a proportion of invariant sites (I), and Γ-distributed rate variation among sites (the most highly parameterized model available in PAUP*) provided the best fit to the data. Parameter estimates for this model obtained for the tree noted above were used in subsequent tree searches, thereby greatly reducing computation time. ML parameter estimates were as follows: base frequencies: A = 0.3889, C = 0.3479, G = 0.099, T = 0.1642; relative transformation rates: A-C = 0.2664, A-G = 4.3169, A-T = 0.3874, C-G = 0.1494, C-T = 4.0770, G-T = 1.0000; proportion of invariant sites: I = 0.4791; shape parameter for the Γ distribution: α = 0.6426. Ten heuristic searches with random addition of taxa were completed for each ML analysis.

RESULTS

Phylogeny

The strict consensus of six most parsimonious trees resulting from an analysis of well-aligned regions only is show in Figure 2. This tree includes a strongly supported sister relationship between the two genera of parasitic finches, *Anomalospiza* and *Vidua*. In addition, monophyly of other in-

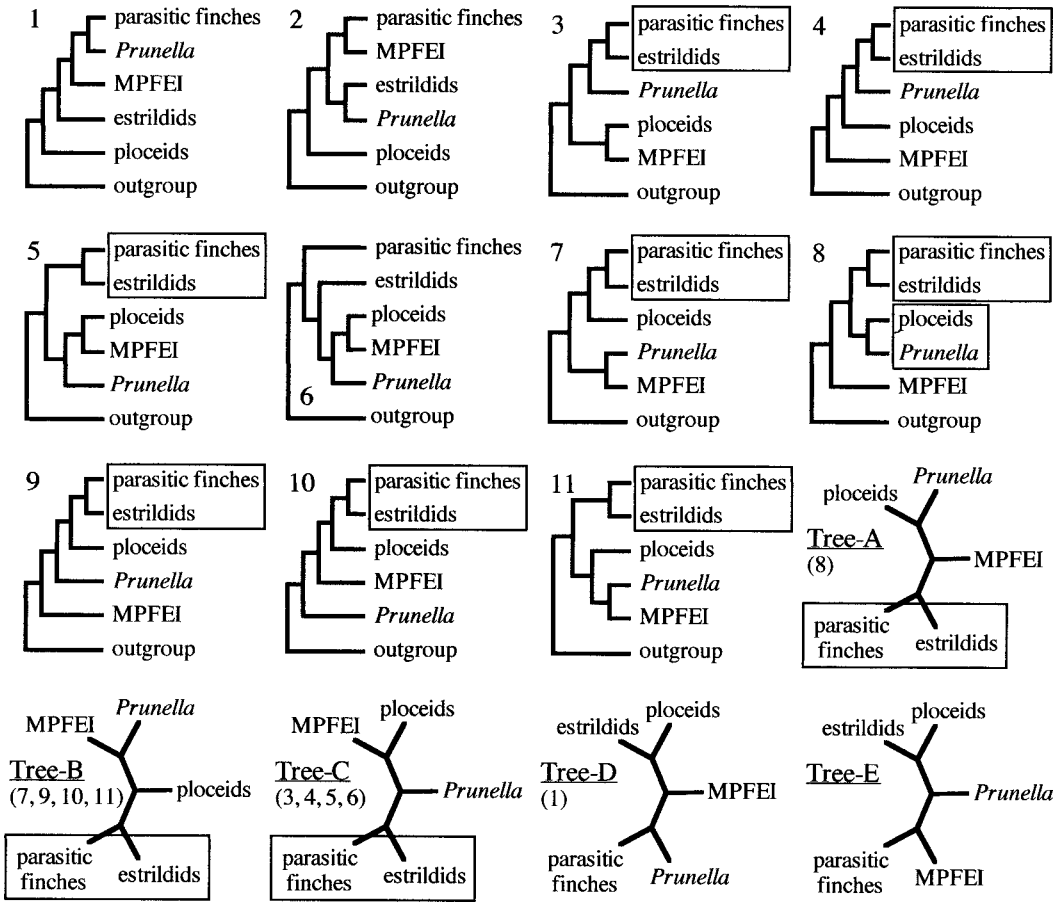


FIG. 3. Alternative phylogenetic hypotheses for the relationships among major clades within the ingroup (see Tables 2, 3, and 4). Only trees found in one or more of our analyses are shown. There are 105 possible rooted trees and 15 possible unrooted trees for the five ingroup clades. With the exception of alternative positions for *Ambylospiza*, monophyly of these five clades was supported in all analyses. Note that many of the rooted trees have identical ingroup topologies: T3, T4, T5, and T6 have the same ingroup topology, equivalent to tree C; similarly, T7, T9, T10, and T11 all have ingroup topology B.

group families is generally well supported, with the exception of an unexpected placement of *Amblyospiza*, a ploceid, near the base of the tree. Also well supported is a clade including representatives of Motacillidae, Passeridae, and the New World nine-primaried oscines (including Fringillidae, Emberizidae, and Icteridae). This clade was also found by Groth (1998). A sister relationship between *Prunella* and the parasitic finches is very weakly supported as are other basal nodes within the ingroup. Among the outgroup taxa, *Eremophila*, *Pycnonotus*, *Elminia*, and representatives of Sylviidae fall within a single clade. This grouping is particularly unexpected for *Elminia*, which is one of the five Corvida outgroup taxa.

Analyses on different partitions of the dataset including or excluding gap regions and using a variety of weighting schemes all support monophyly of the parasitic finches. Our remaining analyses therefore focus on identifying the sister group of the parasitic finches, while exploring the information content of gap regions and the effect of divergent base composition on our analyses. To facilitate comparison of results from different analyses, Figure 3 shows alternative hypotheses for the relationships among five clades within our ingroup: parasitic finches, estrildids, ploceids, *Prunella*, and a clade (''MPFEI'') comprising the remaining ingroup taxa (see Fig. 2).

The results of parsimony analyses using well-aligned regions only and with varying degrees of transition down-weighting are summarized in Table 2. We found different tree topologies depending on whether *Amblyospiza* was included or excluded for less extreme weighting schemes, but similar results when transitions were down-weighted by 40% or more. With *Amblyospiza* included, estrildid finches are the sister group of parasitic finches when transitions are down-weighted by 40% or more. In addition, the position of *Amblyospiza* shifts from the base of the tree to a sister relationship with *Sturnus* (10–30% down-weighting of transitions) and then to a position within a monophyletic Ploceidae (40–100% down-weighting). Similarly, *Elminia* shifts to the base

TABLE 2. Effects of transition down-weighting on tree topology for analyses of well-aligned regions only. The basic topology(ies) obtained in each analysis is indicated by a number (e.g., T1) corresponding to trees in Figure 3. Note that T3, T4, T5, and T6 all have the same ingroup topology (tree C in Fig. 3).

Down-weighting of transitions (relative tv:ts weights)	All taxa	*Amblyospiza* excluded
0% (1:1)	T1[1]	T7
10% (1.1:1)	T2[2]	T7
20% (1.25:1)	T1, T2[2]	T7
30% (1.3:1)	T2[2]	T7
40% (1.7:1)	T3[3]	T4
50% (2:1)	T3[3]	T4
60% (2.5:1)	T3[3]	T4
70% (3.3:1)	T4[3]	T4
80% (5:1)	T4[3]	T3, T4
90% (10:1)	T4[3]	T3, T4
100% (1:0)	T3, T4, T5, T6[3]	T3, T4

[1] *Amblyospiza* at base of tree as in Figure 2.
[2] *Amblyospiza* sister to *Sturnus vulgaris.*
[3] *Amblyospiza* within a monophyletic Ploceidae.

of the tree, grouping with the other Corvida, with 40–100% down-weighting of transitions. With *Amblyospiza* excluded from the analysis, estrildids are the sister taxon of parasitic finches regardless of weighting scheme, but the sister group of the estrildids plus parasitic finch clade changes from ploceids (T7) to *Prunella* (T3, T4) as transition weight is decreased.

Transversion weighting reduces the influence of transitions, which typically are assumed to provide less reliable information about relationships because they accumulate much more rapidly than transversions in mitochondrial sequences (e.g., Brown et al. 1982; Wakeley 1996). The unexpected placement of *Amblyospiza* in our equal-weights analysis is not, however, simply due to excessive homoplasy obscuring the historical signal or to long-branch attraction between *Amblyospiza* and the outgroup due to an accelerated rate of change in this lineage (Felsenstein 1978; Hendy and Penny 1989). Rather, *Amblyospiza* shows a systematic difference in base composition bias as compared to other taxa (Fig. 4A). *Amblyospiza* has the highest proportion of adenine (A), the lowest proportion of cytosine (C), and the second lowest proportion of guanine (G) among the 30 ingroup taxa. Three of the five Corvida outgroup taxa also have a low proportion of C. The unusual base composition in *Amblyospiza* is evident in all three gene regions sequenced, in all three codon positions in ND2 and ND6, respectively, and in both stem and loop regions of RNA genes (data not shown). There is a larger number of nucleotide positions at which *Amblyospiza* has, respectively, A or T and other ploceids have G or C than vice versa, suggesting that directional biases in A-G and C-T transitions in *Amblyospiza* are different than in other ploceids. The *Amblyospiza* lineage also appears to have had a higher rate of C-to-A than A-to-C transversions. Down-weighting of transitions therefore reduces but does not eliminate the influence of the divergent base composition in *Amblyospiza*, perhaps accounting for different tree topologies in analyses with and without this taxon (Table 2).

Analysis of the full dataset using optimization alignment in POY yielded trees of length 9401 and higher. Table 3

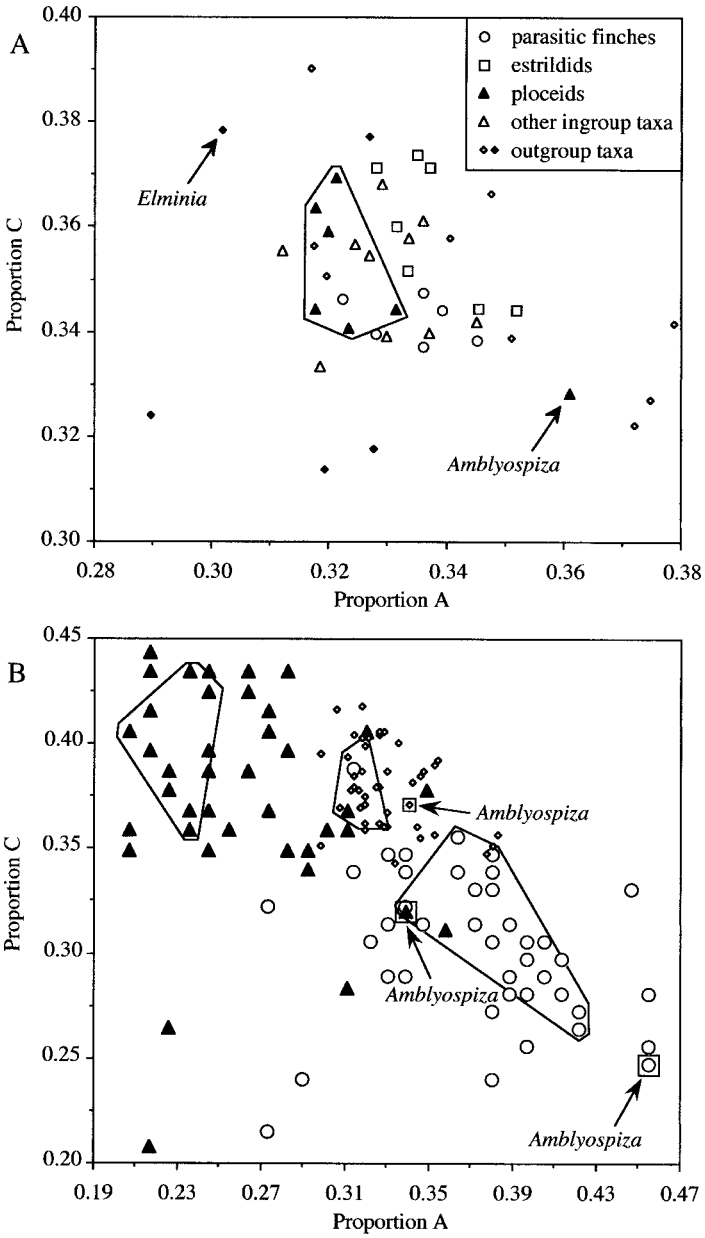


FIG. 4. (A) Base composition at variable positions in ND2 and 12S (ND6, tGlu, and control region were excluded because these data were not available for all taxa). Each point represents base composition for a single taxon. We plot proportions of adenine (A) and cytosine (C) because they show the greatest variation among taxa. *Amblyospiza* also has the second lowest proportion of guanine (G) among the taxa in our study, while its proportion of thymine (T) is just slightly above average. Datapoints for the other six ploceids are enclosed in a polygon. (B) Base composition in parsimony informative ND2 and 12S characters partitioned into three subsets. Each point represents the base composition for a single taxon for one of three subsets of characters: (1) triangles: 106 characters that have fewer steps on tree 1 (Table 3) with basic topology T1 (Fig. 3); (2) circles: 121 characters that have fewer steps on tree 4 (Table 3) with basic topology T8 (Fig. 3); and (3) diamond: 572 characters with the same number of steps on both trees. Points for *Amblyospiza* are inside boxes and indicated by arrows. Points for the other six ploceids are enclosed in polygons. Positions with a gap in one or more taxa were excluded.

TABLE 3. Results of optimization alignment analyses on the full dataset with equal weights for all changes. The six best trees found using POY are listed along with their original and revised scores (see Materials and Methods). The best tree for each criterion is indicated in bold. Tree 4 is shown in Figure 5.

		Tree length				
Tree	Basic topology	Original POY score	Revised POY score	Final static alignment	Well-aligned regions	Gap regions
1	T1[1]	**9401**	9392	(9427)[3]	**7784**	(1608)[4]
2	T8[2]	9402	9394	9386	7800	**1586**
3	T8[2]	9402	9392	9386	7791	1595
4	T8[2]	9404	9391	**9380**	7792	1588
5	T8[2]	9404	9394	9388	7799	1589
6	T8[2]	9404	**9390**	9384	7792	1592

[1] *Amblyospiza* at base of tree as in Figure 2.
[2] *Amblyospiza* sister to *Prunella*.
[3] This value is greater than the original or revised POY scores because the static alignment was optimized on trees with basic topology T8 rather than T1.
[4] Based on revised POY score rather than final static alignment.

provides information on the six best trees found in 200 replicate searches with random addition of taxa. One of these trees has the same basic topology (T1) as the equal-weights trees for well-aligned regions only (as in Fig. 2). The other five trees include a sister relationship between estrildids and parasitic finches and a sister relationship between *Prunella* and *Amblyospiza*, with this group sister to the other ploceids (T8). By independently optimizing each of 25 separate gap regions on these trees (see Materials and Methods), tree scores were reduced by eight to 13 steps as compared to the original scores output by POY. Further trial and error adjustment of the implied alignments generated by POY resulted in an additional reduction of six to 11 steps for the five trees with basic topology T8. A final score of 9380 for tree 4 (Table 3) was 24 steps shorter than the original POY score for this tree. Although another alignment may have a lower score on this or a different topology, this alignment-topology combination (Fig. 5) is the most parsimonious explanation we found for our entire dataset under the criterion of equal weights for all changes. By comparison, the shortest tree found using an alignment constructed by Clustal W (Thompson et al. 1994) is 366 steps longer than our best POY-based alignment, representing an 4% increase in tree length overall and a 23% increase for gap regions.

Improvements in the alignment beyond the revised score in Table 3 highlight the fact that it is impossible to be sure that any one tree topology is really more parsimonious than another. With gap regions included, topologies T1 and T8 have essentially the same score (Table 3), but very different placements of *Amblyospiza* and different sister groups for the parasitic finches. We suggest that this result is not due to a lack of phylogenetic signal in the data, but instead reflects a balance between two large, conflicting sets of characters that differ in base composition (Fig. 4B). Characters with fewer steps on T8 have a higher proportion of A and a lower proportion of C, a bias in the same direction as the base composition shift in *Amblyospiza*. In other words, at positions with a tendency toward greater A and less C content (i.e., positions in which ploceids have base composition more similar to *Amblyospiza*), there is greater support for placing *Amblyospiza* in the ingroup and near ploceids. In contrast, positions with an overall tendency toward less A and greater C content tend to place *Amblyospiza* with the outgroup.

Interestingly, gap regions provide greater support for T8 than T1, whereas well-aligned regions slightly favor T1 (Table 3). Gap regions comprise only 11% of the dataset, but account for 20% of phylogenetically informative characters and 17% of tree length. As optimized on the tree in Figure 5, gap region characters actually have higher consistency and retention indices (CI and RI, respectively) than characters in well-aligned regions (gap regions: CI = 0.42, RI = 0.56; well-aligned regions: CI = 0.28, RI = 0.39), in part because there are fewer steps per variable character (4.7 vs. 5.7) but also because there is, on average, a larger number of states per character in gap regions. In addition, Bremer support indices and bootstrap values based on our best topology-alignment combination indicate that including gap regions substantially increases character support for many nodes (cf. Fig. 5 with Fig. 2).

Bremer support indices in Figure 5 should be interpreted as follows: The Bremer index for a given node is the number of additional steps in the shortest tree without that node in an analysis using a static alignment that was obtained by optimizing sequences on the best tree. This provides a measure of character support for each node in that particular alignment. It is also reasonable to ask how many additional steps are needed in the shortest topology-alignment combination that does not include a particular node. To answer this, the alignment is optimized on trees inconsistent with a particular node to find the shortest solution. This procedure necessarily yields Bremer support indices equal to or smaller than those based on a static optimized alignment.

Because optimization alignment maximizes character support for the best tree, it could be argued that even random data in gap regions will contribute to the support for a particular tree because the alignment has been optimized on that tree. If gap regions contribute historical information that is largely consistent with the rest of the dataset, however, then support indices for most well-supported nodes calculated in the second manner above should be greater than those obtained for well-aligned regions only. Unfortunately, given heuristic estimation of tree length in POY (and therefore a lack of precise comparability of tree scores) and the time required for optimization alignment tree searches, calculating support indices in this manner is problematic. We therefore focussed on the two key nodes in our analysis, the parasitic finch clade and the estrildid plus parasitic finch clade. Using 100 replicate tree searches in POY, we searched for the shortest topology-alignment combinations inconsistent with each of these nodes (to save time, analyses included the 30 ingroup taxa only). Using this approach, the parasitic finch clade has a Bremer support index of 31, as compared to 20 for well-aligned regions only and 36 for the static alignment as optimized on the tree in Figure 5. Similarly, the Bremer support index for the estrildid plus parasitic finch clade was 8, as compared to 5 for well-aligned regions only and 11 for the static alignment. These comparisons suggest that gap regions contribute phylogenetic information to the analysis and do not simply conform to the best topology for well-aligned regions.

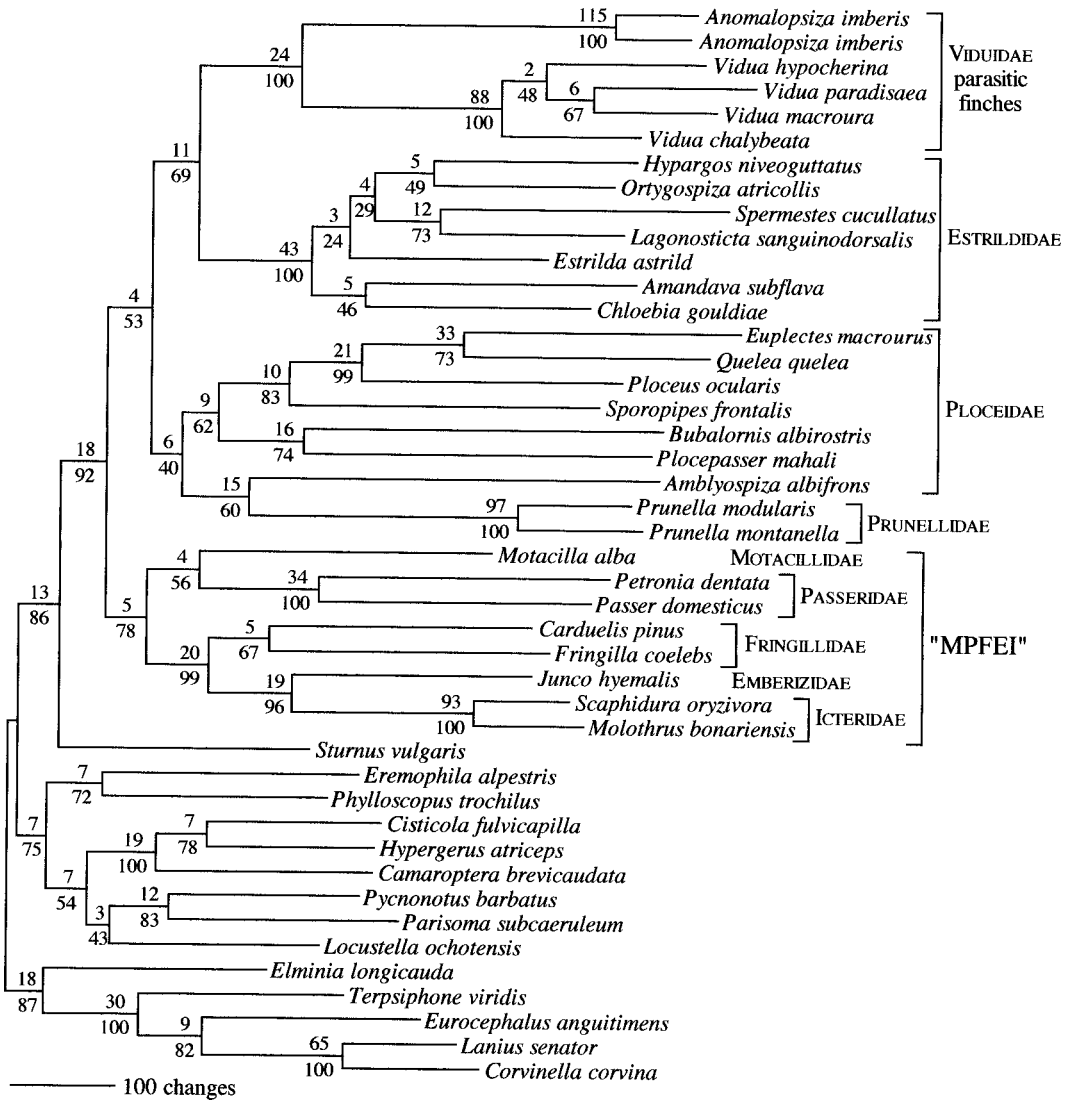


FIG. 5. Most parsimonious tree found using optimization alignment on the full dataset and equal weights for all changes. The tree with the lowest revised score is shown (tree 4, Table 3). Tree length = 9380; CI = 0.30; RI = 0.42. Bremer support indices and bootstrap values calculated in the context of our best static alignment are shown above and below nodes, respectively. Branch lengths are proportional to the number of parsimony-inferred changes. Note that branches are shorter for the outgroup taxa because ND6, tGlu, and the control region were not sequenced for these samples.

Similar considerations apply to analyses using different weighting schemes or different sets of included taxa. A comparison of tree topologies found using optimization alignment in POY with those obtained using a static alignment demonstrates that optimizing the alignment on one particular tree influences the outcome of analyses with different weighting schemes or different sets of included taxa (Table 4). For example, the sister relationship between *Prunella* and *Amblyospiza* found in an equal-weights analysis of the full dataset (Fig. 5) is also found in weighted analyses when the alignment has been optimized on the equal-weights tree (see results under Static Alignment in Table 4). With *Amblyospiza* excluded, the basic topology remains the same, with *Prunella* as the sister taxon of ploceids (T8). In contrast, when the alignment is reconsidered in analyses with *Amblyospiza* excluded, *Prunella* is never found to be the sister taxon of ploceids (see results under Best POY Solution in Table 4). In addition, all optimization alignment analyses, regardless of weighting scheme, agree on the same ingroup topology (tree B) when *Amblyospiza* is excluded. Notably, this is a different topology than obtained in most analyses on well-aligned regions only (see results under Gap Regions Excluded in Table 4 and results in Table 2). Finally, a sister relationship between the parasitic finches and estrildid finches is supported in all but two of the analyses summarized in Table 4 (both involving equal weights analyses of well-aligned regions only).

ML analyses were also sensitive to the inclusion or exclusion of *Amblyospiza*. With *Amblyospiza* included, ML analysis returned a tree with topology T8 (Fig. 3), but with *Amblyospiza* sister to *Prunella*, the same basic topology found in the equal-weights parsimony analysis of the full dataset

TABLE 4. Comparison of tree topologies obtained with gap regions excluded, the static alignment as optimized on the tree in Figure 5, and optimization alignment searches in POY with different weighting schemes and sets of taxa. Note that tree A has the same ingroup topology as T8. Similarly, tree B is consistent with T7, T9, T10, or T11, all of which have the same ingroup topology (see Fig. 3), and tree C is consistent with T3, T4, T5, and T6. All of these analyses include a sister relationship between parasitic finches and estrildid finches, except for equal weights analyses with 44 or 29 taxa and gap regions excluded.

	Gap regions excluded	Static alignment	Best POY solution
44 taxa			
Equal weights	T1[1]	T8[3]	T8[3]
50% (2:1)	T3[2]	T8[3]	T8[2]
20% (5:1)	T4[2]	T8[3]	T8, T10[2]
ML	T8[3]		
43 taxa (*Amblyospiza* excluded)			
Equal weights	T7	T8	T7, T9
50% (2:1)	T4	T8	T10, T11
20% (5:1)	T3, T4	T8	T9
ML	T4		
30 ingroup taxa			
Equal weights	tree B[3]	tree A[3]	tree A[3]
50% (2:1)	tree C[2]	tree A[3]	tree B[3]
20% (5:1)	tree C[2]	tree A[3]	tree B[4]
ML	tree C[2]		
29 ingroup taxa (*Amblyospiza* excluded)			
Equal weights	tree D, E	tree B	tree B
50% (2:1)	tree C	tree B	tree B
20% (5:1)	tree C	tree B	tree B
ML	tree C		

[1] *Amblyospiza* at base of tree as in Figure 2.
[2] *Amblyospiza* within a monophyletic Ploceidae.
[3] *Amblyospiza* sister to *Prunella.*
[4] Ploceids paraphyletic.

in POY (Fig. 5, Table 4). With *Amblyospiza* excluded, ML analysis returned topology T4, including a sister relationship between *Prunella* and the estrildid plus parasitic finch clade. This topology (T4, tree C) was found in many of the weighted parsimony analyses of well-aligned regions only, but differs from the topology generally found in parsimony analyses that included gap regions (tree B, see Tables 2, 4). One other notable difference between ML and parsimony analyses was that the ML topologies included a monophyletic Sylviidae (Old World warblers).

Testing Alternative Hypotheses

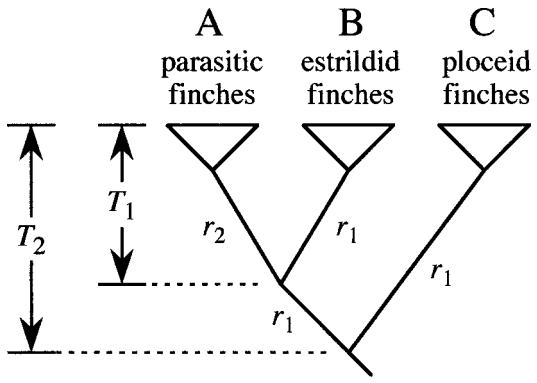
We tested two alternative phylogenetic hypotheses for the parasitic finches by searching for the shortest tree(s) consistent with specified constraints (Table 5). We completed these tests in three different ways: (1) using only well-aligned portions of the dataset; (2) using the full dataset with the alignment optimized on the tree in Figure 5; or (3) using constrained tree searches in POY in which the alignment was optimized on the best tree consistent with the alternative hypothesis. Trees in which *Anomalospiza* is sister to or within Ploceidae and in which *Vidua* is sister to estrildids (as in Fig. 1) require 30, 58 and 41 additional steps, respectively, using the three methods above. Similarly, 13, 29, and 16 additional steps are needed in the shortest trees in which the parasitic finches (both *Vidua* and *Anomalospiza*) are with the ploceids rather than the estrildids. The intermediate number of extra steps for analyses in POY indicates that gap regions contribute to the evidence against these alternative hypotheses even when gap region alignments are optimized on the best topology consistent with the alternative hypothesis. In the context of our best static alignment, both of these alternative hypotheses require a significant increase in tree length (see Table 5 for details).

Antiquity of Parasitic Behavior

The divergence time between a parasitic clade and the most closely related nesting lineage provides an estimate of the maximum time since the evolution of parasitic behavior, whereas the basal split within a parasitic clade provides an estimate of the minimum time since the evolution of parasitism. To compare the relative ages of the parasitic finches and the New World cowbirds, we added to our data matrix ND2 sequences for 32 icterids from Johnson and Lanyon (1999). A parsimony analysis with a total of 76 taxa and 50% down-weighting of transitions yielded 12 trees of length 6989.5. Using the strict consensus of these trees, we calculated the average genetic distance between each group of parasitic birds and their respective sister group and tested for differences in evolutionary rates between groups following the methods of Steel et al. (1996; M. Steel, pers. comm. 1997). We used genetic distances based only on transversion

TABLE 5. Tests of alternative phylogenetic hypotheses. The best tree(s) consistent with the phylogenetic hypothesis presented in Figure 1 or monophyly of a parasitic finch plus ploceid finch clade is compared with the best unconstrained tree(s). Two-tailed *P*-values are from the test described by Templeton (1983) as implemented in PAUP* (Swofford 2000). A small *P*-value suggests that the alternative hypothesis requires a significant increase in tree length as compared to the unconstrained tree. Where multiple equally parsimonious trees were found, the range of *P*-values for all comparisons between the best constrained and best unconstrained topologies is given.

	Increase in tree length required to obtain alternative hypothesis			
	Equal weights parsimony		Transitions down-weighted 50%	
Hypothesis	Extra steps	*P*-value	Extra steps	*P*-value
1. ((*Vidua*, Estrildidae), (*Anomalospiza*, Ploceidae))				
Gap regions excluded	30	0.043–0.080	29	0.008–0.018
All characters (static alignment)	58	0.0008	53	0.0001
All characters (POY)	41		41.5	
2. (*Vidua*, *Anomalospiza*, Ploceidae)				
Gap regions excluded	13	0.36	8.5	0.079–0.22
All characters (static alignment)	29	0.027	22	0.019–0.041
All characters (POY)	16		11	



$$d_{AC} = T_2 r_1 + (T_2 - T_1) r_1 + T_1 r_2 = 0.0925$$

$$d_{BC} = 2T_2 r_1 = 0.0738$$

$$d_{AB} = T_1 (r_1 + r_2) = 0.0798$$

setting $r_1 = 1$,

$$T_2 = d_{BC} / 2r_1 = 0.0369$$

$$T_1 = \frac{d_{AB} + d_{BC} - d_{AC}}{2r_1} = 0.0306$$

$$r_2 = \frac{d_{AB} + d_{AC} - d_{BC}}{2T_1} = 1.61$$

FIG. 6. Logic and equations used to calculate the rate of sequence evolution in parasitic finches relative to that of ploceids. It is assumed that the parasitic finch lineage has had a constant rate of evolution (r_2) since its divergence from the estrildid lineage and that a second rate of evolution (r_1) applies to the rest of the tree. Empirical values for genetic distances (d_{AB}, d_{AC}, d_{BC}) are the average, uncorrected transversion distances between clades. Times (T_1, T_2) are expressed as transversions per nucleotide position per lineage (for a lineage evolving at rate r_1).

differences, which appear to have a nearly linear accumulation through time over relatively long time scales (Sheldon et al. 2000; van Tuinen et al. 2000).

Relative rates tests indicate that the parasitic finches have an accelerated rate of sequence evolution, which complicates a comparison of the relative ages of the two parasitic clades. For the ND2 gene, *Vidua* and *Anomalospiza* are 25% more divergent from ploceids than are estrildid finches and this difference in evolutionary rate is highly significant ($Z = 3.89$, $P < 0.001$). Analyses excluding either the parasitic finch clade or the estrildid clade, respectively, demonstrate that this difference is due primarily to an accelerated rate of evolution in parasitic finches rather than a reduced rate in estrildids. When parasitic finches are excluded, evolutionary rate does not differ between estrildids and ploceids ($Z = 1.02$, $P > 0.2$); but when estrildids are excluded, the rate in parasitic finches is significantly faster than in ploceids ($Z = 3.87$, $P < 0.001$). Assuming one rate of evolution in estrildids and ploceids and a different rate in parasitic finches and using logic similar to that of Li et al. (1981), we calculate that the rate of ND2 sequence evolution has been 61% faster in parasitic finches than in estrildids (Fig. 6). ND2 showed the greatest difference in rates, but for the complete dataset (including ND2, 12S, ND6, and the CR), the parasitic finch clade is evolving 50% percent faster than the estrildid finch clade ($Z = 4.83$, $P \ll 0.001$). No difference in rate was found between the parasitic cowbirds and their sister group ($Z = 0.12$, $P > 0.8$).

After correcting for the higher rate of sequence evolution in parasitic finches, sequence divergence between parasitic finches and estrildids is still greater than that between the parasitic cowbirds and their sister group (Table 6), suggesting a longer history of brood parasitism in African finches than in New World cowbirds. The divergence between *Anomalospiza* and *Vidua* is 7.6 times as old as the basal split within the parasitic cowbird clade. Alternatively, if we assume that parasitic behavior evolved at the midpoint between the minimum and maximum estimates for each parasitic clade, then parasitic behavior is 4.4 times older in African finches than in cowbirds. The split between *Anomalospiza* and *Vidua* (0.053 transversion differences per site, after adjusting for the faster rate in parasitic finches) is also older than the deepest divergence among the estrildid finches sampled here (0.043 transversion differences per site).

Converting these genetic distances into absolute time estimates is problematic given the lack of relevant fossil or biogeographic information with which to calibrate evolutionary rates for the taxa in our study. Recent studies on songbirds have used the conventional 2% divergence per million years for mtDNA (Klicka and Zink 1997, 1999; Avise et al. 1998), although Fleischer et al. (1998) estimated the rate of cytochrome *b* evolution in Hawaiian drepanids to be only 1.6%. For the taxa in our analysis, uncorrected ND2 genetic distances are 6.5 times as large as transversion distances for pairwise comparisons in which taxa are 7% divergent or less (i.e., those comparisons in which saturation

TABLE 6. Relative estimates of the minimum and maximum age of brood parasitic behavior in the two lineages of brood parasitic songbirds. Time is expressed as ND2 transversion distance (transversions per site) per lineage. Time estimates for the parasitic finches have been adjusted downward to account for the faster rate of sequence evolution in this group (see Fig. 6).

	Average transversion distance ± SD	Relative time since divergence
Maximum estimates		
Parasitic finches vs. estrildids	0.0798 ± 0.006	0.0306
Parasitic cowbirds vs. sister clade	0.0189 ± 0.003	0.0095
Minimum estimates		
Anomalospiza vs. *Vidua*	0.0855 ± 0.008	0.0266
Molothrus rufoaxillaris vs. other parasitic cowbirds	0.0071 ± 0.002	0.0035

should have relatively little effect). Multiplying transversion distance by 6.5 suggests an overall genetic divergence between estrildids and parasitic finches of 51.9%, which when adjusted for the higher rate in parasitic finches becomes 39.8% (see Fig. 6). Assuming an evolutionary rate of 2% sequence divergence per million years (or, equivalently, a transversion rate of 0.31% divergence per million years), parasitism in African finches evolved sometime between 17 million and 20 million years ago. By the same logic, parasitism in cowbirds evolved between 2 million and 6 million years ago.

Discussion

Phylogenetic Inferences

Our analysis of mitochondrial sequence data yields three key results, each of which lends insight into the evolution of brood parasitism and coevolution between parasitic finches and their hosts. First, *Vidua* and *Anomalospiza* are sister taxa and therefore obligate brood parasitism evolved only once in Old World finches. In particular, *Anomalospiza* is not closely related to ploceids, as suggested by its other name, the parasitic weaver. This reduces by one the tally of avian lineages that have evolved obligate brood parasitism. Second, parasitic finches represent an ancient lineage that is clearly distinct from other groups of Old World songbirds. Third, parasitic finches appear to be most closely related to estrildid finches, which include all the host species parasitized by *Vidua*. Although all of our analyses strongly support the first two results, identification of the sister group of parasitic finches was less clear. A clade comprising parasitic finches and estrildids was obtained, however, in all analyses that included gap regions (Table 4). ML analyses, down-weighting of transitions and/or exclusion of *Amblyospiza* from the dataset also yielded trees in which estrildids are sister to parasitic finches (Tables 2, 4).

We suggest that divergent base composition in one ploceid, *Amblyospiza*, strongly affected the overall tree topology, including the sister group of parasitic finches, in our initial analysis of well-aligned regions only (Fig. 2). Two sets of conflicting characters reflect convergence in base composition between *Amblyospiza* and the outgroup on one hand and the common ancestry of ploceids on the other (Fig. 4B). Down-weighting transitions mitigated this effect to some degree but both weighted parsimony and ML analyses were still sensitive to the inclusion or exclusion of *Amblyospiza* (Table 4).

Lineage-specific changes in base composition represent a kind of nonindependent character evolution that violates the assumptions of both parsimony and ML analyses and can mislead analyses employing even the most sophisticated models of sequence evolution (Naylor and Brown 1998). Our analyses suggest that base composition issues are important in analyses of closely related genera and families in addition to those involving major taxonomic lineages (e.g., Naylor and Brown 1998; Foster and Hickey 1999).

We think it is interesting that including gap regions tipped the balance in our equal-weights parsimony analyses toward a solution in which *Amblyospiza* was within the ingroup, consistent with a variety of behavioral and morphological evidence (Collias and Collias 1964; Crook 1964; Bentz 1979) and in which estrildids were sister to parasitic finches (Table 3). Gap regions presumably evolve under somewhat different constraints than other portions of the mitochondrial genome, and *Amblyospiza* was less divergent from other ploceids in gap region base composition than in the rest of the dataset.

We used optimization alignment (as implemented in POY) to maximize use of the phylogenetic information present in gap regions in the most objective way possible. Optimization alignment tree searches constrained to find alternative topologies (Table 5) indicate that gap regions provided real historical information that was generally consistent with the rest of the dataset rather than just conforming to the best tree for well-aligned regions. Although Wheeler (1996) originally suggested that optimization alignment could result in solutions with fewer steps than possible in a conventional alignment, our success in deriving a static alignment with fewer steps than the best solution provided by POY suggests that any optimization alignment solution based on simple gap costs can be represented as a static alignment with gap characters. Using this optimized static alignment for subsequent analyses based on the optimal tree (such as the determination of Bremer support indices) is a reasonable way to evaluate character support inherent in that particular alignment. It is also clear, however, that optimizing the alignment on one particular tree will bias analyses using different weighting schemes or different subsets of taxa toward finding similar topologies (Table 4). Thus, the alignment should be reconsidered in any new tree search that involves a change in the taxa or gene regions included and/or a change in weighting scheme.

Additional tests of phylogenetic hypotheses for Old World songbird families should reconsider relevant behavioral and morphological characters and employ different kinds of molecular data, such as nuclear coding or intron sequences, which may be less affected by differences in base composition among taxa. Our analyses suggest that a sister relationship between parasitic finches and estrildid finches is the best hypothesis given the available data, and we discuss the implications of this result in the following sections.

Origin of Parasitic Behavior

Possible starting points for the evolutionary transition from nesting to obligate brood parasitism include facultative parasitism, communal laying within a cooperative breeding system, and the takeover or use of nests built by other species (Payne 1977, 1998b). All of these behaviors are observed in Old World songbirds, but to varying degrees in different groups. Both cooperative breeding and conspecific nest parasitism are more frequent in ploceids than estrildids (Crook 1958; Lewis 1982; Dhindsa 1983a, 1990; Freeman 1988; Jackson 1993), whereas certain *Passer* species frequently use other species' nests (Summers-Smith 1988) and *Prunella* is known for its polygynandrous breeding systems (Davies 1992; Heer 1996). The sister relationship with estrildids, however, favors nest takeover as the most likely precursor to parasitism in parasitic finches. At least 20 estrildid species use nests built by other species (e.g., van Someren and van Someren 1945; Chapin 1954; Immelmann et al. 1965, 1977;

Immelmann and Immelmann 1967; Goodwin 1982; Restall 1997; Payne et al. 2002; Payne 2002) and several African and Asian estrildids (*Amadina* spp., *Euodice cantans*, *Estrilda malabarica*, *Clytospiza*, *Nesocharis*, *Amadina subflava*, *Lagonosticta nitidula*) use old weaver nests more often than they build their own. Alternative models for the origin of parasitism, however, are not mutually exclusive: At least two estrildids engage in both intraspecific parasitism and nest takeover (Dhindsa 1983b; Birkhead et al. 1990). In Indian silverbill (*E. malabarica*), for example, more than one female sometimes lays in nests that are appropriated from other species (Dhindsa 1983b). Similar behavior might have led to the evolution of obligate parasitism in the common ancestor of *Vidua* and *Anomalospiza*.

Host-Parasite Coevolution

The sister relationship between *Vidua* and *Anomalospiza* and, in turn, between the parasitic finch clade and estrildids allows for two equally parsimonious explanations for derived behavioral and morphological characters shared by *Vidua* and their estrildid hosts, but not by *Anomalospiza* and other Old World finches. Such characters either evolved independently in *Vidua* and estrildids or they evolved once in the common ancestor of parasitic finches and estrildids and were then subsequently lost in *Anomalospiza*. Characteristics in *Vidua* that mimic those of their hosts might therefore be explained either by selection for mimicry in the context of host-parasite coevolution or more simply by common ancestry. Parsimony reconstruction of discrete character states does not lead to a preference for one explanation over the other, but other considerations are relevant. If loss of complex traits is generally more likely than gain, then traits shared by *Vidua* and estrildids may be attributed to shared ancestry. This reasoning is also conservative with respect to making inferences that coevolution has produced convergent similarities in parasites and hosts. In addition, loss of certain traits in *Anomalospiza* is consistent with the apparent switch by this lineage from relatively closely related hosts (as in parasitism of estrildids by *Vidua*) to relatively distantly related Old World warblers, represented by *Cisticola fulvicapilla* in our analyses (see Fig. 5), although this particular species is not known to be a host (Friedmann 1960; Payne 1997b, 2002). Finally, ancestral character states may have been intermediate between the alternatives observed today, allowing for other evolutionary scenarios (see below).

In addition to mouth markings and gape papillae that mimic those of their hosts, *Vidua* young resemble estrildids in their unusual begging and feeding behavior. Like estrildids, young *Vidua* beg by twisting their heads nearly upside down and waving both head and tongue from side to side, rather than by stretching upward. In response, host parents regurgitate seeds from the crop into the mouths of the young. These behaviors limit *Vidua* to parasitizing estrildid finches with similar behavior (Nicolai 1964; Kunkel 1969; Payne et al. 2001; Payne and Payne 2002). In contrast, young *Anomalospiza* and the young of their warbler hosts are fed directly with insects and neither host nor parasite have mouth markings with contrasting color patterns or gape elaboration. In addition, young *Anomalospiza* beg much like other small birds and without the twists and turns seen in *Vidua* (Roberts 1917; Friedmann 1960; Steyn 1996).

We suggest that the earliest brood-parasitic finches parasitized closely related species with mouth markings and begging behavior similar to their own. Genes inherited from a common ancestor for mouth colors, spots, and gape papillae would have provided both a starting place for parasitic young to succeed in the nests of a related host species as well as the genetic basis for the later development of species-specific mimicry. Mouth markings and begging behavior characteristic of estrildids and *Vidua* were presumably lost in the lineage leading to *Anomalospiza* after it switched to parasitizing more distantly related hosts, perhaps as a direct result of coevolution with these new hosts. The gain and loss of these characteristics, however, is not necessarily an all or none question. As noted by Kunkel (1969, p. 179), ''The morphological and ethological characters used in the begging and feeding of Estrildids are so peculiar that the Viduines would never have succeeded to imitate them if they had not begun to parasitize these birds before the full development of these patterns.'' In other words, ancestral begging behavior and mouth markings may have been simpler and more generalized than those observed in extant species, and the elaboration of these characters may have proceeded in parallel in host and parasitic clades. An origin of brood parasitism in African finches that predates the deepest split within the estrildid clade (see Results) is consistent with this hypothesis. That ancestral estrildids were very likely parasitized prior to their dispersal to Asia and Australia also allows for the possibility that elaborate mouth markings and begging behavior originated in the context of a coevolutionary arms race between host and parasite. The continued maintenance of these traits in species not subject to parasitism, however, argues for other explanations (e.g., parent-offspring signaling; Payne 1997a; Kilner 1999; Payne et al. 2001).

Although our analysis suggests that similarities between *Vidua* and their hosts can be attributed to ''community of descent'' (Friedmann 1960), the remarkable host specificity of mouth markings in many *Vidua* species is almost certainly due to subsequent selection in the context of host-parasite coevolution (Payne 1967, 1977). Host-specific mimicry of juvenile plumage in a few *Vidua* species (Payne and Payne 1994, 2002; Payne 1997b) also suggests evolutionary specialization, whereas shared ancestry is a sufficient explanation for the white eggs shared by all *Vidua* and estrildids (Roberts 1939). The eggs of *Anomalospiza* vary in ground color and are generally marked (Friedmann 1960; Vernon 1964), reflecting divergence from the ancestral state for parasitic finches and perhaps coevolution with their hosts. As in other brood parasitic birds (Kilner et al. 1999), the begging calls of *Anomalospiza* and most *Vidua* do not precisely mimic those of their hosts (Vernon 1987; Payne et al. 1998; Payne and Payne 2002).

The males of most *Vidua* species mimic the songs of their host species (Nicolai 1964, 1973; Payne 1973, 1982, 1998b; Payne and Payne 1994, 1995). Males learn songs from their foster parents and other birds (including other male *Vidua* reared by the same host species; Nicolai 1973; Payne et al. 1998). Song learning is widespread among songbirds (Kroodsma and Baylis 1982) and has been observed in es-

trildids that were cross-fostered in captivity (Goodwin 1960, 1971, 1982; Güttinger 1972, 1973; Immelmann 1972, 1975; Baptista 1973; Clayton 1989). Thus, song copying or imitation probably did not arise de novo in *Vidua* but developed from behavioral mechanisms present in the common ancestor of estrildids and parasitic finches. *Anomalospiza* has songs unlike those of its hosts (Pakenham 1939; Williams and Keith 1962; Payne 1997b), suggesting that song learning mechanisms were modified in this lineage after its switch to more distantly related hosts.

Recurrent Speciation in Parasitic Finches?

Song learning and behavioral imprinting appear to have played a central role in the diversification of extant *Vidua* species and in the development of species-specific associations with hosts. When reared by a novel host in captivity, male village indigobirds (*V. chalybeata*) imprint on the new host and learn their songs (Payne et al. 1998). Female indigobirds also imprint on the novel host and as adults choose both to mate with males mimicking songs of the new host and to parasitize nests of the new host (Payne et al. 2000). In nature, these behavioral mechanisms contribute not only to the persistence and specialization of *Vidua* species on individual hosts but also to the establishment of new, reproductively isolated parasite populations when new hosts are colonized (Payne 1973; Payne et al. 1992, 2002; Payne and Payne 1994, 1995). Very limited mitochondrial differentiation among 10 morphologically distinct indigobird species suggests a recent and rapid radiation of these forms associated with the colonization of new host species (Klein and Payne 1998; M. D. Sorenson and R. B. Payne, unpubl. data).

The above model of recent speciation in indigobirds contrasts strikingly with the ancient origin of brood parasitism that is indicated by the deep split we found between *Vidua* and *Anomalospiza*. If *Vidua* and *Anomalospiza* diverged 17 million years ago (see Results), then the most recent common ancestor of extant *Vidua* dates to less than 4 million years ago. Likewise, the most recent mitochondrial ancestor of all 10 indigobird species dates to less than 500,000 years ago, with the species within each geographic region forming much more recently (M. D. Sorenson and R. B. Payne, unpubl. data). We suggest that parasitic finches and their estrildid hosts have had a long but extremely dynamic coevolutionary history in which extant *Vidua* represent only the most recent iterations of an ongoing process of host colonization, speciation, and extinction in the parasitic lineage. Although ancestral *Vidua* very likely parasitized ancestral estrildids and mimicked their mouth markings, host-specific mimicry has probably evolved repeatedly as new hosts were colonized (or old hosts were recolonized), rather than during an ancient and restricted process of host-parasite cospeciation (Payne 1998a).

If this kind of dynamic process has been repeating itself since the origin of parasitism in African finches, it provides a potential explanation for our finding of a faster rate of sequence evolution in this group. Hypotheses for rate variation among taxa that involve differences in metabolic rate (and correlated variation in body size and/or generation time; e.g., Martin and Palumbi 1993; Nunn and Stanley 1998) are not likely to apply to parasitic finches and their hosts because they share a recent common ancestor, similar body size, and life-history parameters (Payne 1973, 2002). An alternative explanation for differences in evolutionary rate is the greater fixation of nearly neutral mutations in lineages experiencing small population size (Ohta 1992). Parasitic birds in general have much smaller populations than their hosts (Payne and Payne 1977) and, if the above model of speciation is correct, parasitic finches would have experienced repeated bottlenecks associated with host switching during their long history as brood parasites. For the coding regions in our dataset, the parasitic finch lineage is not only evolving more rapidly than estrildids or ploceids (see Results) but also has experienced a slightly higher ratio of nonsynonymous to synonymous substitutions, as would be predicted if the parasitic finches lineage has had a higher fixation rate for nearly neutral mutations (see Ohta 1992). We used the program MEGA (Kumar et al. 1993) to estimate the number of synonymous (d_S) and nonsynonymous (d_N) substitutions per site for 505 codons in ND2 and ND6. The means for eight pairwise comparisons of d_N and d_S between *Vidua* and *Anomalospiza* were 0.110 and 0.794, respectively, yielding a ratio (d_N/d_S) of 0.139. For 49 comparisons between estrildids and ploceids, the means were 0.078 (d_N) and 0.853 (d_S), yielding a ratio of 0.092. Similar results have been reported for the endosymbiotic bacteria of aphids as compared to free-living forms with much larger populations (Moran 1996; Wernegreen and Moran 1999). Larger molecular datasets are needed to test whether an accelerated rate of sequence evolution is a general result for parasitic birds.

Conclusions

Although somewhat unexpected given the morphological and behavioral divergence between *Anomalospiza* and *Vidua*, our analysis leads to the clear conclusion that these two genera are sister taxa and that obligate brood parasitism had a single and ancient origin in African finches. In addition to highlighting the relative rarity of this behavior among birds (Davies 2000), this results indicates a very long history of host-parasite coevolution, but one in which associations between specific hosts and parasites have not been continuous. The effect of long-term coevolution between host and parasite clades should be considered in the ongoing debate regarding historical (evolutionary lag) versus adaptive (evolutionary equilibrium) explanations for the apparent lack of effective defenses against parasitism in many host species (e.g., Lotem et al. 1992; Soler et al. 1998; Takasu 1998; Robert and Sorci 1999; Rothstein 2001). In ancient parasitic clades, one also might predict the evolution of flexible genetic architectures controlling traits such as egg markings in cuckoos and mouth markings in *Vidua* that might facilitate the rapid evolution of mimicry following host switches.

Our analyses also suggest that *Vidua* finches are relatively closely related to their hosts, as appears to be the case for other avian parasites (i.e., cowbirds; honeyguides, family Indicatoridae; black-headed duck, *Heteronetta atricapilla*). The origin of obligate parasitism in all these groups likely involved parasitism of closely related host species that accepted and provided appropriate care for parasitic young. A sister

relationship with estrildids also suggests that nest takeover was the behavioral precursor to the evolution of parasitism in finches. A similar explanation may apply to honeyguides (which are related to cavity-nesting woodpeckers and barbets) and to cowbirds, but intraspecific parasitism may have been the starting point for cuckoos and the black-headed duck (Davies 2000).

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