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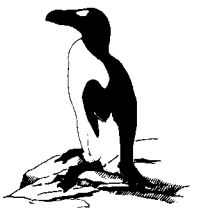

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PHYLOGENETICS OF DARWIN'S FINCHES: PARAPHYLY IN THE TREE-FINCHES, AND TWO DIVERGENT LINEAGES IN THE WARBLER FINCH

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ABSTRACT.—The Galapagos Darwin's finches (Geospizinae) have been classified as three major groups based on morphology and behavior: ground-finches, tree-finches, and the Warbler Finch (*Certhidea olivacea*). Little is known about the evolutionary relationships within and among these groups, which is partly due to the lack of a phylogeny based on molecular sequence data. We used mitochondrial sequence data to reconstruct a phylogeny of Darwin's finches. These data show that within the tree-finches, only one genus is conclusively monophyletic, and another is conclusively paraphyletic. It may be appropriate to uphold the classification of the tree-finches into two genera. The Warbler Finch complex is paraphyletic, as revealed by two divergent genetic lineages contained within this species. Stochastic lineage sorting within relatively recently diverged species and interspecific and intergeneric hybridization are the two most likely explanations for the sharing of haplotypes among taxa.

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ADAPTIVE RADIATION refers to the process in which one species evolves into numerous species over a relatively short period of time. The question of how this occurs is fundamental to studies of evolution and speciation and has been at the heart of considerable research (e.g. DeSalle et al. 1987, Sang et al. 1994, Losos 1995, Tarr and Fleischer 1995, Cameron et al. 1996, Radtkey 1996, Shaw 1996). Perhaps the most famous ongoing study of adaptive radiation involves Darwin's finches (Geospizinae). More than 100 years of research have been conducted on Darwin's finches, yet, many questions about their evolutionary history remain unanswered. A glaring omission in this field of study is the lack of a sequence-based phylogeny. A molecular data set for the group will yield further insight into a number of aspects of evolution and speciation, including adaptive radiation.

The subfamily Geospizinae comprises 14 nominate species, 13 of which inhabit the Galapagos Archipelago. Darwin's finches have been divided into three groups based on morphology and behavior: ground-finches, tree-finches, and the Warbler Finch (*Certhidea olivacea*; Table 1). The ground-finches (*Geospiza*) comprise one genus and six species that are finch-like in appearance, particularly with respect to their bills, and spend much of their time foraging on the ground. The tree-finches (*Camarhynchus, Platyspiza,* and *Cactospiza*) comprise six species, but the number of genera oscillates between one and three (see below). These six species have bills intermediate to those of ground-finches and the Warbler Finch, and although they occasionally forage on the ground in a manner similar to ground-finches, they spend much of their time in foliage and vegetation exhibiting behavior similar to that

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TABLE 1. Darwin's finch species, taken from Lack (1947), Bowman (1961), and Grant (1986). Authors for species names are given in Lack (1947), although some generic names of tree-finches differ from this reference.

Scientific name	English name
Ground-Finches	
Geospiza magnirostris Gould[a]	Large Ground-Finch
Geospiza fortis Gould[a]	Medium Ground-Finch
Geospiza fuliginosa Gould[a]	Small Ground-Finch
Geospiza difficilis Sharpe[a]	Sharp-beaked Ground-Finch
Geospiza scandens (Gould)[a]	Cactus Ground-Finch
Geospiza conirostris Ridgway[a]	Large Cactus Ground-Finch
Tree-Finches	
Camarhynchus parvulus (Gould)[a]	Small Tree-Finch
Camarhynchus pauper Ridgway	Medium Tree-Finch
Camarhynchus psittacula Gould[a]	Large Tree-Finch
Platyspiza crassirostris Gould[a]	Vegetarian Finch
Cactospiza pallida (Sclater and Salvin)[a]	Woodpecker Finch
Cactospiza heliobates (Snodgrass and Heller)	Mangrove Finch
Warbler Finch	
Certhidea o. olivacea Gould[a]	Warbler Finch (Santiago, Santa Cruz, Isabela, Ferdinand, Rabida, Seymour, Pinzon islands)
C. o. bifasciata	Warbler Finch (Santa Fe Island)
C. o. fusca[a]	Warbler Finch (Pinta and Marchena islands)
C. o. luteola	Warbler Finch (Floreana Island)
C. o. ridgwayi	Warbler Finch (San Cristobal Island)
C. o. becki	Warbler Finch (Wolf and Darwin islands)
C. o. mentalis[a]	Warbler Finch (Genovesa Island)
C. o. cinerascens[a]	Warbler Finch (Española Island)

[a] Taxa used in this study.

of the Warbler Finch. The Warbler Finch is a monotypic genus consisting of eight subspecies. True to its name, this species closely resembles a warbler with respect to its small size, slender bill, and habit of gleaning animal food from foliage.

Morphological, behavioral, and allozyme data (Lack 1947, Yang and Patton 1981, Schluter 1984) all agree with the division of the Galapagos finches into the three groups outlined above. However, little is known about the phylogenetic associations among the groups, and even less is known about the relationships of genera, species, and subspecies within each group. The only published genetic studies of Darwin's finches are based on allozyme data (Ford et al. 1974, Yang and Patton 1981, Polans 1983) that lack the level of resolution necessary to infer many phylogenetic relationships. In this paper, we use mitochondrial sequence data to clarify unanswered questions pertaining to the phylogeny of Darwin's finches.

First, we address evolutionary relationships of the three groups. Existing phylogenies consistently treat the Warbler Finch as the basal taxon, but the positions of the tree-finches and ground-finches remain equivocal. The latter groups generally are treated as monophyletic sister groups (Lack 1947, Schluter 1984); however, it has also been suggested that the tree-finches are ancestral to the ground-finches (Stern and Grant 1996), and under this scenario it is possible that the tree-finches are a paraphyletic group. Prior to investigating evolutionary relationships within the tree-finches, we wished to ascertain whether taxonomic separation of the tree-finches and ground-finches, based on morphological data, was reflected by their DNA sequences. Because Darwin's finches underwent adaptive radiation relatively recently (Yang and Patton 1981), there may have been insufficient time for complete lineage sorting to have occurred following speciation, which could result in the sharing of haplotypes among species (Neigel and Avise 1986). In addition, hybridization has been documented in Darwin's finches (Grant 1986), and this may lead to introgression of mitochondrial haplotypes from one species to another (Tegelström 1987). We reconstructed a phylogeny of the

three groups using mitochondrial sequence data, which allowed us to infer relative levels of genetic relatedness within and among the tree-finches, ground-finches, and the Warbler Finch.

The second question we address pertains to the taxonomy of the tree-finches. Using morphological and behavioral data, Lack (1947) placed all tree-finches in *Camarhynchus,* although he later modified this and placed *Platyspiza crassirostris* (Vegetarian Tree-Finch) in a monospecific genus (Lack 1969). This classification has been upheld by Schluter (1984), again based on morphological data. More common in recent literature is the division of the tree-finches into the genera *Camarhynchus, Platyspiza,* and *Cactospiza,* as shown in Table 1. All six species appear to be closely related, as evidenced by the fact that allozyme data were not sufficiently differentiated to resolve relationships at either the genus or species level (Yang and Patton 1981). We used sequence data to clarify classification of the tree-finches.

The third question is directed at the Warbler Finch, which possibly is the most enigmatic species in terms of its historical classification. Gould (1837) included the Warbler Finch in the first comprehensive description of Darwin's finches, but Darwin and other taxonomists questioned the validity of classifying it as a finch. Subsequent to Gould's treatise, *Certhidea* was reclassified as a member of various other families, including the wood-warbler family Parulidae (formerly the Mniotiltidae; Darwin 1841, Ridgway 1897, Snodgrass and Heller 1904). Since the turn of the century, most taxonomists have agreed with the placement of *Certhidea* in the Geospizinae (e.g. Lowe 1936, Lack 1947, Tordoff 1954, Yang and Patton 1981, Schluter 1984).

The Warbler Finch inhabits all major islands and a few of the minor islands of the Galapagos (Lack 1947, Harris 1973, Grant and Schluter 1984, Grant 1986). The eight subspecies are differentiated largely on the basis of plumage color (Lack 1947, Bowman 1961, Lack 1969). The genetic relationships among the *Certhidea* subspecies have not been adequately investigated. Two biochemical studies have tentatively concluded that the Santa Cruz population differs from a group containing the Marchena, Española, and Genovesa populations (Ford et al. 1974, Polans 1983). A third study found that populations from Santa Cruz, Marchena, Española, and Genovesa had diverged from one another (Yang and Patton 1981). All of these conclusions remain equivocal, because sample sizes were very small. Our goal was to ascertain the phylogenetic relationships of the subspecies.

Methods

The phylogeny of the three groups was reconstructed from a combination of 16S rRNA and control-region sequences from representatives of the ground-finch, tree-finch, and Warbler Finch groups. We also included two mainland species, the Black-faced Grassquit (*Tiaris bicolor*) and the Bananaquit (*Coereba flaveola*), that were designated as outgroups. These outgroups were chosen because they have been suggested as close relatives of the Geospizines (Harris 1973, Bowman 1983, Baptista and Trail 1988). For our second phylogeny, we amplified and sequenced a portion of the control region from 20 tree-finch and Warbler Finch species and subspecies, and reconstructed a phylogeny of these individuals using the Bananaquit as the outgroup.

Amplification.—DNA was extracted from approximately 10 μL of blood using 500 μL of 5% Chelex 100 (BioRad, Hercules) in ddH_2O, following the manufacturer's protocol. All polymerase chain reactions were done in a Perkin Elmer 9600 thermocycler, using 3 μL of extracted DNA in a total volume of 100 μL, with 0.5 U *Taq* polymerase and 1X react buffer (Gibco BRL), 2 mM $MgCl_2$, 200 μM dNTPs, and 1 μM of each primer. Amplification primers were GSL Glu (5′-TTGGTTGTAACTTCAGGAAC-3′)and 12 sr (5′-AAGGTTAGGACTAAGTCTTT-3′) for the control region (H. Gelter unpubl. data), and 16SL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SH (5′-CGGTCTGAACTCAGATCACGT-3′) for 16S rRNA (Palumbi et al. 1991). The parameters were one cycle of 94°C for 2 min; 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; and one cycle of 72°C for 10 min. The amplification products were precipitated with 250 μL of 95% ethanol and 20 μL of linear polyacrylamide and then resuspended in 15 μL ddH2O. The resuspended samples were harvested from a 0.8% agarose gel with 1X TBE buffer using a QIAEX (QIAGEN) kit and eluted in 24 μL of ddH_2O.

DNA sequencing.—All sequencing was done following a double-stranded dideoxy sequencing protocol, using primers GSL 148 (5′-CCCTATTCTCATTATTTTCGGC-3′), GSL 248 (5′-TATGAATCCCCTAACACCCAG-3′), and CR 367 (5′-TAGTGTAATGGTTGCCGGAC-3′) for the control region and 16SL, 16SH (sequences shown above), and 16SL2 (5′-TCTCTTACAGGCAATCGGTG-3′) for 16S rRNA. A Sequenase 2.0 kit (United States Biochemical) and deoxyadenosine-5′-triphosphate [alpha-S35] were

employed with the following deviations from the Sequenase USB protocol: (1) the primer and template annealing reaction included 1 μL of 5% NP40, 1 μL DMSO, 1 μL of 10 μM primer, 7 μL DNA, and 2 μL 5X Sequenase reaction buffer per sample; (2) during the primer and template annealing step, the samples were boiled for 3 min, placed in liquid nitrogen for 3 min, and then allowed to warm up to 15°C; (3) the labeling reaction included 1.3 μL ddH2O, 1 μL Mn buffer, and 1 μL 5% NP40; and (4) the termination reaction was incubated for 4 min at 42 to 44°C. After sequencing, the samples were run on a 35 × 45 cm 6% polyacrylamide gel for 2 and 4.5 h at 60 watts. We dried the gels in a BioRad Model 583 gel dryer and then exposed the sequences to Kodak Biomax film.

Data collected for the phylogeny of the three major groups comprised a combination of 361 base pairs (bp) of 16S rRNA and 304 bp of control region (665 bp total) from two populations of Warbler Finch (*Certhidea o. fusca* and *C. o. cinerascens*), six ground-finches (*Geospiza difficilis, G. scandens, G. magnirostris, G. fortis, G. fuliginosa,* and *G. conirostris*), four tree-finches (*Camarhynchus parvulus, C. psittacula, Cactospiza pallida,* and *Platyspiza crassirostris*), the Black-faced Grassquit, and the Bananaquit. The data for the phylogeny of the tree-finch genera and the Warbler Finch subspecies comprised 385 bp of control region from 20 tree-finches and Warbler Finches and a Bananaquit (Table 1, Appendix 1). Sequences from each species were deposited in GenBank (accession numbers AF089768 to AF089795)

Sequence alignment and phylogenetic analysis.—Gels were scored manually, and sequences were aligned using GeneWorks (IntelliGenetics, Inc.). Few insertions/deletions (gaps) occurred, but the gene alignment program never had to insert more than one gap at any given site to achieve a plausible alignment. Sequence divergence was calculated using the number of nucleotide differences between two sequences, including gaps. Sequence divergence equals the number of nucleotide differences divided by the total number of nucleotides in the sequence, expressed as a percentage.

Sequence alignments from GeneWorks were imported into PAUP (Swofford 1993) and PHYLIP (Felsenstein 1993). Transitions and transversions were equally weighted because owing to the low frequency of transversions (see Results), differential weighting did not affect tree topologies. Because the control region and 16S rRNA sequences are not protein-coding, we did not differentiate between synonymous versus nonsynonymous substitutions when analyzing these sequences. To circumvent the dilemma of differential weighting of gaps, we did two analyses that led to inferred maximum-parsimony trees: (1) one with all gaps treated as informative sites, and (2) one with all gaps treated as missing data. Maximum-parsimony trees were generated in PAUP (Swofford, 1993). Neighbor-joining (using the Kimura two-parameter distance measure) and maximum-likelihood (using empirical base frequencies and a single substitution rate category) trees were generated in PHYLIP (Felsenstein, 1993).

RESULTS

The first data set provided 23 phylogenetically informative sites (18 excluding the outgroups) and 44 variable sites (22 excluding the outgroups). Twenty percent of the nucleotide substitutions were transversions, but only 5% of these involved comparisons within the Darwin's finches. Some taxa had a single deletion. Only two different sequences were found in all six species of ground-finches. The sequence divergence was 0.2 to 2.4% within the Darwin's finches and 2.6 to 3.8% between the Darwin's finches and the outgroups (Table 2). The relatively close relationship among the outgroups and the Geospizinae justifies the choice of outgroup. Maximum-parsimony (Fig. 1), maximum-likelihood (not shown), and neighbor-joining (not shown) trees showed the same topology. In addition, the same topology resulted when gaps were treated as either informative or missing sites, and when the transition/transversion ratio was set to either 1.0 or 4.0.

The phylogeny shows that the Warbler Finches are the sister group to the other Darwin's finches. The ground-finches form a well-supported clade within the cluster of tree-finches and ground-finches, demonstrating that tree-finches are more closely related to one another than they are to ground-finches. Therefore, we can investigate the relationships among tree-finches in isolation from ground-finches. We also have demonstrated the suitability of using the Warbler Finch as an outgroup versus the ground-finches or tree-finches.

The second data set yielded 15 control-region haplotypes, which in conjunction with the outgroup had 35 variable sites, 18 of which were phylogenetically informative (Table 3). These values were 21 and 17, respectively, when the outgroup was not included. The amount of sequence divergence among haplotypes, including the outgroup, ranged from 0.25 to 3.6%. All nucleotide substitutions among the Warbler Finch and tree-finches were transitions. Two of the Warbler Finch haplotypes had a gap at one or two of the sites. The maximum-parsimony tree (Fig. 2), maximum-likelihood analysis (not

TABLE 2. Variable sites in 361 bp of 16S rRNA and 304 bp of control-region sequence; "*" denotes an insertion/deletion in the sequence, and "-" indicates identity with the first sequence.

Species	Control region	16S rRNA
G. difficilis	A T T T T A G A A G A A T T T A A T T T A T C A * G A A A T T C	A A C A A C T G T C T T
G. scandens	- C - - C -	- - - - - - - - - - - -
C. parvulus	- C - C C - - - - - G - - - - - - - C - - - - - - - - - - - C -	- - - - - - - - - - - A
C. psittacula	- C - - C - - - - - G - - - - - - - C - - - - - - - - - - - C -	- - - - - - - - - - - -
C. pallida	- C - - C - - - - - G - - - - - - - C - - - T - - - - - - - C -	- - - - - - - - - - - -
P. crassirostris	- - - - - G - - - - G - - - - - - - C - - - - - - - - - - - C -	- - - - - - C - - - - -
C. olivacea fusca	G C - - C - A - - - - G - - - - - - - - G - - - T - * T - C C -	G - - G G - C A - - - A
C. o. cinerascens	G C - - C - A - - - - G - - - - - - - - G - - - T - * T - C C -	G - - G G - C A - - - -
T. bicolor	G C C - C - - G T - - - - - - T T - C - - C - T - - * T - - C -	- - T - - A C - C - C -
C. flaveola	G C - - C G - - - A - - C G A - T G C A G - - - - T * T T - - T	- G - - - A C - - A C -

shown), and neighbor-joining analysis (not shown) recovered the same topology. Treating the gaps as either missing data or as informative sites did not affect the topology.

The tree-finches are presented as a monophyletic group relative to the Warbler Finch, regardless of whether the Bananaquit or a Warbler Finch subspecies is designated as the outgroup. *Platyspiza crassirostris* is the only species of tree-finch that appears to be monophyletic. *Camarhynchus psittacula* contains one individual that is allied with *Cactospiza pallida* and one individual that has the same haplotype as *Camarhynchus parvulus.* These levels of cohesion are reflected in a comparison of within- and among-species sequence divergences. Only *P. crassirostris,* the monophyletic assemblage, has conspecific sequence deviations that are absolutely lower than the heterospecific sequence deviations (Table 4). Overall, the tree-finches are a very closely related group of species.

The Warbler Finch is divided into two distinct lineages according to the islands that each inhabits (Fig. 3): (1) the Santa Cruz clade (*C. o. olivacea*) and (2) the Marchena, Española, and Genovesa (M-E-G) islands clade (*C. o. fusca, C. o. mentalis,* and *C. o. cinerascens*). The control-region sequence divergence within each of these two Warbler Finch clades is 0 to 0.7%, whereas the divergence between clades is 2.0 to 2.7%. The Warbler Finches are presented as a paraphyletic group regardless of whether the outgroup comprises only the Bananaquit, or the Bananaquit plus the Warbler Finches.

DISCUSSION

Relationships of the three groups.—Our results do not disagree with the traditional view (Lack 1947) of the Warbler Finch as the basal taxon and the tree-finches and ground-finches as monophyletic sister groups (Fig. 1). We found no evidence to support Stern and Grant's (1996) proposal that the ground-finches arose from the tree-finches, although as previously stated, these findings must be considered preliminary because the inclusion of alternate or additional individuals may alter the resulting phylogeny. These data do not support Yang and Patton's (1981) suggestion that the tree-finches differentiated more recently than the ground-finches, because the tree-finch and ground-finch mtDNA sequence divergence from the basal

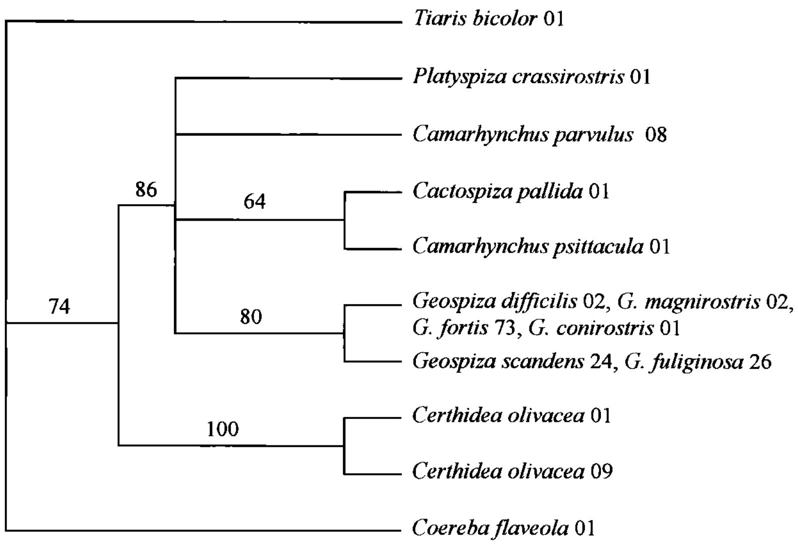

FIG. 1. Maximum-parsimony tree showing the relationships among selected Darwin's finches, based on 665 bp of control region and 16S rRNA sequence. *Tiaris bicolor* and *C. flaveola* are outgroups. Bootstrap values are shown above branches (2,000 bootstrap replicates). Tree length = 53, CI = 0.811. See Appendix 1 for specimen information.

Warbler Finches is 0.9 to 2.6% and 1.1 to 2.3%, respectively. This genetic phylogeny was required to investigate possible instances of morphological convergence or parallelism, because these two phenomena are common in passerines (Bledsoe 1988) and can distort reconstructed phylogenies that are based solely on morphological and behavioral data.

Classification of the tree-finches.—Within the tree-finches, *Platyspiza* is currently classified as a monotypic genus, which is consistent with our results (Fig. 2). In contrast, *Camarhynchus parvulus* does not form a monophyletic group, and the status of *C. psittacula* remains equivocal. The sharing of mitochondrial haplotypes between species may result from ancestral polymorphism and incomplete lineage sorting, or hybridization and introgression (Takahata and Slatkin 1984, Neigel and Avise 1986, Tegelström 1987). When two populations or spe-

TABLE 3. Nucleotide differences between the 15 tree-finches and Warbler Finch mtDNA control-region haplotypes and one *Coereba flaveola* (Cfa) haplotype, based on 385 bp. See Figure 2 for haplotype species identities; "*" denotes an insertion/deletion, and "-" indicates identity with the first sequence.

Haplotype	
H1	G C C A A C G A G T T T A A T T T G C T * * C C C T C G G T C T C A C
H2	- T * - - - - - - - - - - - - -
H3	- - T - - - - G A - - - - - - C - - - - T A T - - C - - - - - - - - -
H4	A - T G G - - G A - - - - - - C - A - - - A T - - - - - - - - - T G T
H5	A - T - G - A G A - - - - - - C - A - - - A T - - - - - - - - - - G T
H6	A - T - G - - G A - - - - - - C - A - - - A T - - - - - - - - - T G T
H7	A - T - G - A G A - - - - - - C - A - - - A T - - - - - - - - - T G T
H8	A - - - G - - G A - - - - - - C - A - - - A T - - C - - - - - - T - T
H9	A - - - G - - G A - - - - - - C - A - - - A T - - C - - - C T - T - T
H10	A T - - G - - G A - - - - - - - - A T - - A T - - C - - - - - - T - -
H11	A T - - G - - G A - - - - - - - - A T - - A T - - C - - - - - - T - T
H12	A T - - - - - G A - - - - - - - - A T - - A T - - C - - - - - - T - T
H13	A T - - G - - G A - - - - - - - - A - - - A T - - C - - - - - - T - T
H14	A - - - G T - G A - - - - - - C - A - - - A T - - C - - - - - - T - T
H15	A - - - G - - G A - - - - - - C - - - - - A T - - C - - - - - - T - T
Cfa	- - - G G - A - A C G A T T G C A - - * T * T T T - T C T - - C - - -

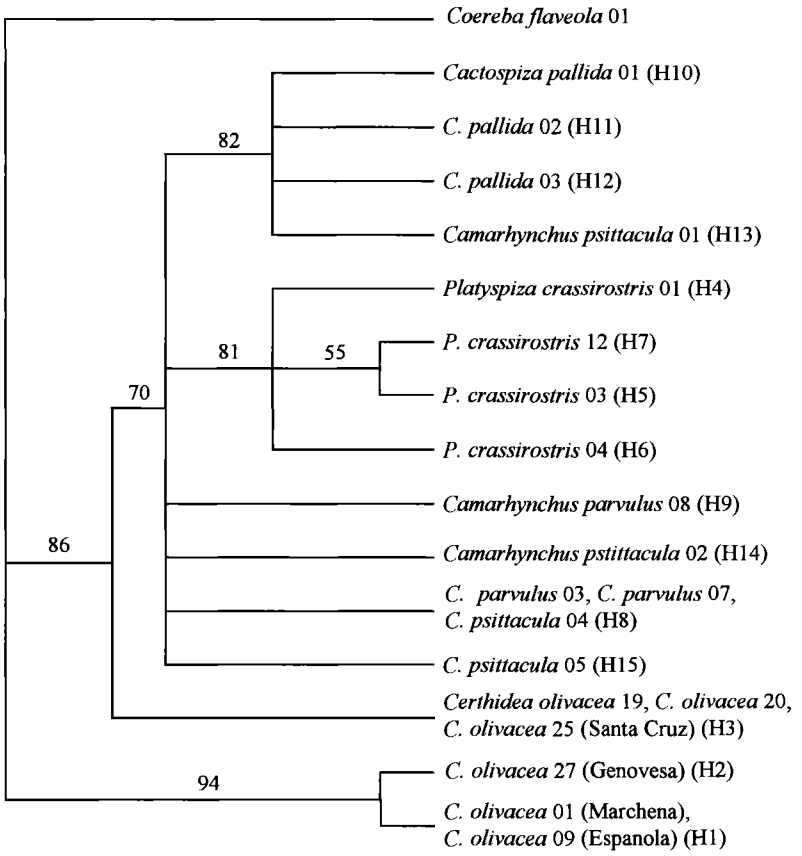

FIG. 2. Maximum-parsimony tree of the tree-finch and Warbler Finch control region haplotypes 1 to 15 (H1 to H15), with *C. flaveola* as the outgroup. Tree length = 81, CI = 0.864. Bootstrap values written above branches (2,000 bootstrap replicates). See Appendix 1 for specimen information.

cies are first separated, their haplotypes are expected to be polyphyletic with respect to one another, based simply on the chance of certain ancestral haplotypes occurring in more than one population or species. Stochastic lineage sorting results in a progression from polyphyly to paraphyly to monophyly (Tajima 1983, Neigel and Avise 1986, Pamilo and Nei 1988). As a result, certain haplotypes will be maintained in a population, and others will go extinct. Barring selection, this is generally a random process. If populations are sampled during the stages of polyphyly or paraphyly, then shared haplotypes may be the result of incomplete lineage sorting (Avise et al. 1983, Moran and Kornfield 1993, Pérez-Suárez et al. 1994).

TABLE 4. Comparison of conspecific and heterospecific sequence divergences among four species of tree-finches.

	Sequence divergences (%)	
	Within species	Among species
Platyspiza crassirostris	0.26 to 0.78	1.00 to 2.3
Camarhynchus parvulus	0.00 to 0.78	0.00 to 2.3
Camarhynchus psittacula	0.26 to 1.00	0.26 to 2.1
Cactospiza pallida	0.26 to 0.52	0.26 to 2.6

Given the close genetic relationships of the tree-finches, it is possible that some individuals in the *Camarhynchus* and *Cactospiza* genera share haplotypes as a result of incomplete lineage sorting. *Platyspiza* is the only tree-finch genus that appears monophyletic in this study, and this seems unlikely to have resulted from either relatively greater age or relatively smaller population size in this species, two factors that can accelerate haplotype lineage sorting

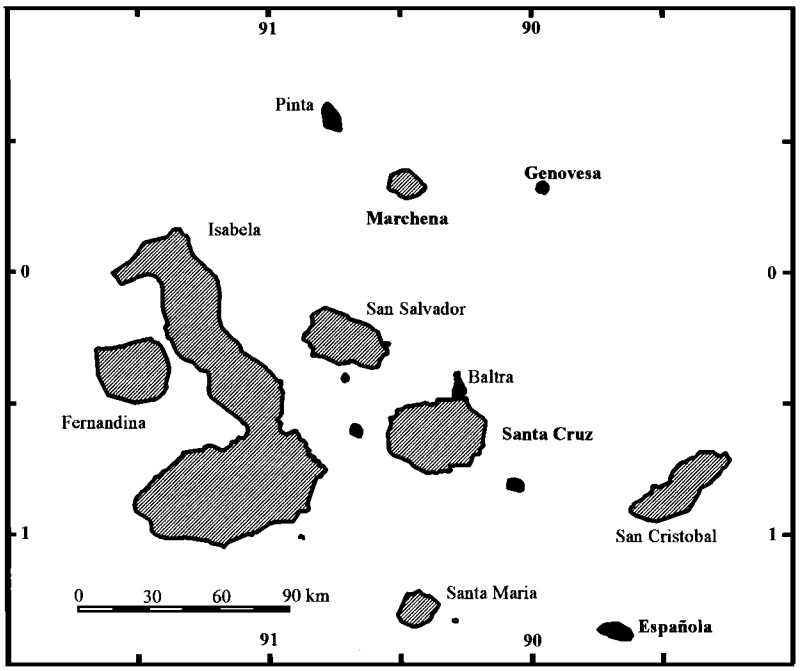

FIG 3. Map of the Galapagos Archipelago. Warbler finch subspecies were sampled from the four islands labeled in bold type (Genovesa, Marchena, Española, and Santa Cruz).

(Avise 1994). *Platyspiza crassirostris* does not appear to be older than the *Camarhynchus-Cactospiza* lineage, because the minimum divergence between *P. crassirostris* and the other tree-finch species is 0.98%. This is considerably lower than the maximum interspecific divergence (1.8%) within the *Cactospiza-Camarhynchus* lineage (Table 4). Furthermore, *Platyspiza, Camarhynchus,* and *Cactospiza* likely had similar long-term population sizes during their evolutionary histories, because population bottlenecks presumably occurred on numerous occasions during the colonization of new islands. In addition, once the islands are colonized, ecological conditions greatly deplete the sizes of established populations in some years (Boag and Grant 1981, Grant and Grant 1992). If we accept that similar evolutionary demographic conditions are likely to have prevailed for all tree-finch genera, then the identification of both monophyletic and paraphyletic genera may be attributed to chance and small sample sizes.

An alternate explanation for the paraphyly of *Camarhynchus* is historic and/or ongoing hybridization between *C. parvulus, C. psittacula,* and *Cactospiza pallida.* This phenomenon is known to occur among the ground-finches and has played a role in the adaptive radiation of that group (Grant 1993, 1994; J. Freeland and P. Boag unpubl. data). Unfortunately, although hybridization among the tree-finches has been documented (Lack 1947, Bowman 1983, Grant 1986), neither the frequency of hybridization nor the relative fitness of hybrids is known. The tree-finches and ground-finches have many similarities, including a high overall sequence similarity within each group, a similar age, the same number of species, and interspecific hybridization (Grant 1986, Yang and Patton 1981, J. Freeland and P. Boag unpubl. data). Although further research is warranted, we suggest that, like in ground-finches, hybridization played a role in the adaptive radiation of tree-finches. The extent of this role remains unclear, and it is extremely difficult with existing data to differentiate between the effects of lineage sorting and hybridization. Based on the mtDNA available sequence data, no basis exists for dividing tree-finches into three genera. The classification of *P. crassirostris* into a monotypic genus is not disputed by our data, but the division of the remaining species into two genera is dubious. Although molecular evidence seldom is considered to be the sole criterion for identifying spe-

cies and genera, it is reasonable to expect two genera to be genetically distinguishable. It is possible that the *Camarhynchus psittacula* individual that is allied with the *Cactospiza pallida* individuals is an anomaly, although given our small sample size, probability dictates that this is unlikely to be the case. Although more individuals must be sequenced before firm conclusions can be drawn, the available genetic data tend to support Lack's (1969) and Schluter's (1984) classification of the tree-finches into two genera (*Camarhynchus* and *Platyspiza*).

The Warbler Finch subspecies.—Not all of the Warbler Finch subspecies are genetically distinct (Fig. 2, Table 3). The Marchena and Española populations share the same haplotype, and their sequence divergence from the Genovesa population is 0.7%. This may result from either retention of an ancestral haplotype, or ongoing gene flow between the Marchena and Española Warbler Finches. Once again, we cannot differentiate between the two processes with certainty, but the probability of the former is inversely proportional to the time since colonization and to the frequency and duration of bottlenecks. Estimates of the time since the tree-finch and Warbler Finch lineages diverged will vary, depending on which populations are compared. The tree-finch/ Santa Cruz Warbler Finch sequence divergence is 1.3 to 2.3%, compared with 2.3 to 3.9% for tree-finch/M-E-G Warbler Finch divergence. The control-region sequence used in this study evolves approximately 2.5 times faster than the rate of cytochrome-*b* and 16S rRNA evolution in Darwin's finches (Freeland 1997), or 5% per million years. If this is true, then the Warbler Finch and tree-finch lineages split about 750,000 years ago. Although this must be treated as a very approximate figure, it falls within the geological ages of the islands, which range from 4 million years to less than 500,000 years (Bailey 1976, Cox 1983). This age also is close to the estimate of 570,000 years since the divergence of *Certhidea* from the other Geospizines based on allozyme data (Yang and Patton 1981).

If the Warbler Finch lineage is between 0.5 to 1 million years old, it seems surprising that the sharing of haplotypes between two populations would be the result of incomplete lineage sorting, because this would mean that the relatively rapidly evolving control region would have remained unchanged in both Marchena and Española for up to 1 million years. In addition, the distinction between the Santa Cruz and the M-E-G Warbler Finches agrees with the tentative findings of Ford et al. (1974) and Polans (1983), suggesting that the pattern of mitochondrial haplotype sharing is similar to the pattern of nuclear differentiation among these four Warbler Finch subspecies. These facts, combined with the regular bottlenecks (Boag and Grant 1981, Grant and Grant 1992) that would have accelerated stochastic lineage sorting, suggest that at some point in their evolutionary history, gene flow played a role in the maintenance of genetic homogeneity among the M-E-G Warbler Finch populations.

The M-E-G Warbler Finches lineage is considerably older than the Santa Cruz Warbler Finch lineage. Both Española and Santa Cruz are among the oldest islands in the archipelago (Cox 1983), and the M-E-G clade may be a relic of one of the first colonizations. The Santa Cruz Warbler Finch population is genetically intermediate to the tree-finches and the M-E-G Warbler Finch clade, and the Warbler Finch subspecies comprise another paraphyletic species. Whereas a number of different scenarios could explain this, the most intriguing possibility is that the original founder population on the Galapagos Islands bore a morphological resemblance to the Warbler Finch. Future work on the comparison of the Geospizinae to mainland species will allow elaboration on this idea.

We acknowledge that the paucity of informative characters limits our conclusions. However, a preliminary investigation into the genetic divergence of Darwin's finches using 16S rDNA, cytochrome *b*, and control-region sequences demonstrated that on the whole the Geospizinae do not comprise a genetically divergent group of birds (Freeland 1997). In all likelihood, genetic differentiation is limited because of recency of speciation (Yang and Patton 1981) and hybridization (Grant 1986, J. Freeland and P. Boag unpubl. data). Therefore, it seems unlikely that more sequencing would clarify phylogenetic relationships. We suggest that future work focus on developing more incisive molecular markers that will show greater differentiation among genera, species, and populations.

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APPENDIX 1. Species identification, showing location and collector or source for each sample.

Species	Site collected[a]	Collector/source[a]
Coereba flaveola 01	Peru	LSU (B5168)
Certhidea olivacea 01	Marchena	G. Seutin
Certhidea olivacea 09	Española	G. Seutin
Certhidea olivacea 19	Santa Cruz (CDRS)	P. Boag
Certhidea olivacea 20	Santa Cruz (CDRS)	P. Boag
Certhidea olivacea 25	Santa Cruz (CDRS)	P. Boag
Certhidea olivacea 27	Genovesa	P. Boag
Cactospiza pallida 01	Santa Cruz (CDRS)	P. Boag
Cactospiza pallida 02	Santa Cruz (CDRS)	P. Boag
Cactospiza pallida 03	Santa Cruz (highlands)	P. Boag
Camarhynchus parvulus 03	Santa Cruz	P. Boag
Camarhynchus parvulus 07	Santa Cruz (CDRS)	P. Boag
Camarhynchus parvulus 08	Santa Cruz (CDRS)	P. Boag
Camarhynchus psittacula 01	Marchena	G. Seutin
Camarhynchus psittacula 02	Marchena	G. Seutin
Camarhynchus psittacula 04	Santa Cruz	P. Boag
Camarhynchus psittacula 01	Santa Cruz (highlands)	P. Boag
Geospiza conirostris 01	Española	G. Seutin
Geospiza difficilis 02	Genovesa	P. Boag
Geospiza fuliginosa 06	Santa Cruz	P. Boag
Geospiza fortis 73	Daphne	P. Boag
Geospiza magnirostris 02	Marchena	G. Seutin
Geospiza scandens 24	Santa Cruz	P. Grant
Platyspiza crassirostris 01	Santa Cruz	P. Grant
Platyspiza crassirostris 03	Marchena	G. Seutin
Platyspiza crassirostris 04	Marchena	G. Seutin
Platyspiza crassirostris 12	Santa Cruz	P. Boag
Tiaris bicolor 01	Unknown	FLMNH (331105)

[a] LSU = Louisiana State Museum of Natural Sciences; CDRS = Charles Darwin Research Station; FLMNH = Florida Museum of Natural History.