

Colonization History of Atlantic Island Common Chaffinches (*Fringilla coelebs*) Revealed by Mitochondrial DNA

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Common chaffinches (*Fringilla coelebs*) are thought to have colonized the Atlantic island archipelagoes (the Azores, Madeira, and the Canaries) from neighboring continental populations (Iberia and north Africa) within the last million years. However, colonization may have occurred separately from north Africa to the Canaries and from Iberia to the Azores (as would be predicted geographically) or in one wave from Iberia to the Azores and then to Madeira and the Canaries. These alternatives have different implications for the evolution of morphometric and plumage differentiation in island chaffinches. To determine the most likely colonization route, we estimated the phylogenetic relationships among island and continental subspecies of common chaffinch using sequences from four mtDNA genes (cytochrome *b*, ATPase 6, NADH 5, and the control region). The most strongly supported mtDNA phylogeny places the continental subspecies together as the sister group to a monophyletic clade containing the island subspecies. This is consistent with a single wave of colonization, and suggests that patterns of similarity among Atlantic island common chaffinches, such as blue pigmentation, short wings, and long tarsi, are due to common colonization history rather than to convergent evolution in a common island environment. However, spectral analysis of phylogenetic splits showed that although monophyly of island haplotypes is favored, there is also substantial support for their polyphyletic origin. We attribute the latter to the confounding effect of homoplasy at multistate sites and to the relatively rapid sequence of colonization events which provided insufficient time for the accumulation of strong phylogenetic signal. These problems are likely to be significant impediments in attempts to test hypotheses of phylogenetic histories of recently evolved populations and taxa. © 1999 Academic Press

INTRODUCTION

Colonization events involving remote oceanic islands provide excellent opportunities to study the processes of microevolution and speciation, particularly when

peripherally isolated populations demonstrate marked divergence relative to their continental counterparts. A classic example of this is afforded by the common chaffinch (*Fringilla coelebs*), a widespread Palearctic passerine species. Within the last million years common chaffinches are thought to have colonized the Atlantic islands (Azores, Madeira, and Canaries) from north Africa (Morocco) and/or Europe (Grant, 1979; Fig. 1). Island populations are characterized by blue dorsal plumage and reddish-orange breasts, similar to extant north African populations. Plumage variation is pronounced enough to warrant recognition of different subspecies in the Azores (*F. c. moreletti*) and Madeira (*F. c. maderensis*), and in the Canaries at least two subspecies can be recognized (*F. c. canariensis* on the middle islands of Gran Canaria, Tenerife, and Gomera, and *F. c. palmae* on the western islands of La Palma and Hierro). Two subspecies have been described from continental north Africa (*F. c. africana* in Morocco and *F. c. spodiogenys* in Tunisia), and the phenotypically distinctive continental European birds have also been accorded separate subspecies status (*F. c. coelebs*). Finally, the congeneric blue chaffinch (*F. teydea*) inhabits the pine forests of Tenerife and Gran Canaria in the Canaries. This distinctive wholly blue species is thought to be the product of an earlier invasion of the Canaries by stock ancestral to the common chaffinch (Stresemann, 1927–1934).

In a major statistical analysis of external measurements taken from museum skins, Grant (1979) demonstrated that island common chaffinches had evolved larger body mass, longer legs and bills, but shorter wings than their continental conspecifics. The greatest shift in morphology was observed in birds from the Azores, which had evolved toward the phenotype of the larger blue chaffinch. Furthermore, beak depth and width had increased on the Azores but not on the Canaries. Grant (1979) interpreted these results as evidence for convergent evolution of chaffinches in separate island environments and possibly character displacement in Canary island populations where blue chaffinches and common chaffinches occur sympatrically. However, an impediment to determining the

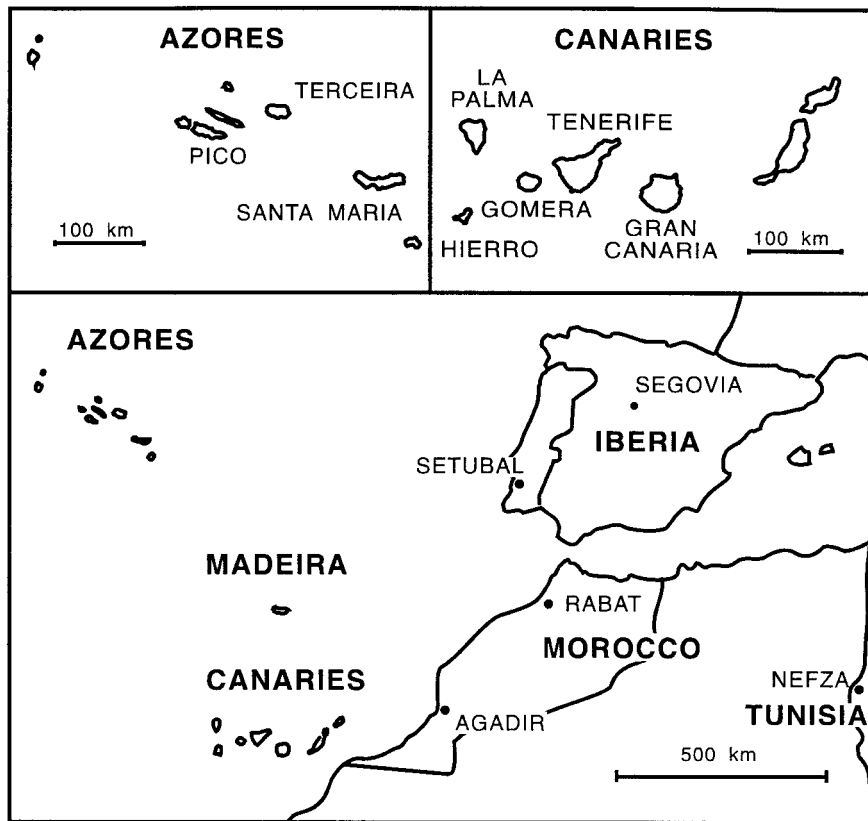


FIG. 1. Map showing common chaffinch subspecies distribution and collection localities.

evolutionary processes responsible for divergence in peripheral isolates arises when their history of colonization has to be inferred from present day distributions (Barton and Charlesworth, 1984). For common chaffinches, Grant's (1979) conclusions derive in part from the assumption that the island archipelagoes were colonized independently from the geographically closest point on the neighboring continents (that is, the Azores were colonized by birds from Iberia and the Canaries by migrants from north Africa). However, alternative source populations exist for each archipelago (Grant, 1980). In particular, a strong case for an Azores origin of Canaries populations can be made on the basis of present day wind patterns, plumage and other morphological characteristics, and examples in other species (Grant, 1980).

Rather than making inferences from environmental, geographical, and ecological factors, an alternative approach to determining the colonization history of a series of peripheral isolates is to examine the genealogical relationships among them. In this context, a population shares a more recent common ancestor with its source population than with other geographically proximal populations. For common chaffinches, we can potentially distinguish an Azores origin for Canary island chaffinches from an independent north African origin if

Azores, Madeira, and Canaries subspecies are monophyletic with respect to continental subspecies. Additionally, if Azores chaffinches subsequently spread to the Canaries via Madeira, then subspecies from the latter two archipelagoes will be most closely related and will be a sister group to the Azores subspecies.

The goals of the present study are to estimate the phylogenetic relationships among subspecies of common chaffinches, to infer the colonization history of chaffinches in the Atlantic islands, and to reexamine the conclusions drawn from previous studies. While morphological characters can be used to generate phylogenies, we have chosen to address this problem using DNA sequence data from several mitochondrial genes. Mitochondrial DNA (mtDNA) has been used widely in phylogenetic studies of recently evolved taxa because it is easily isolated and assayed, is uniparentally inherited, and evolves rapidly (Brown, 1983; Avise *et al.*, 1987), thus providing an abundance of polymorphic characters which are largely unaffected by selection (but see Ballard and Kreitman, 1995). Additionally, because its effective population size is one-quarter that of a nuclear autosomal gene, mtDNA has a substantially higher probability of accurately retrieving short internodes than do nuclear DNA markers (Moore, 1995).

Although evolutionary tree-building methods have become widely accepted, assessing the accuracy of the estimated phylogeny remains problematic. In particular, sampling error (Felsenstein, 1985), inappropriate tree selection criteria (Steel *et al.*, 1993), or incorrect choice of evolutionary model (Olsen, 1987) may lead to an inaccurate phylogeny. To address these problems, we evaluated different tree construction methods combined with bootstrapping to detect sampling error, and we attempted to adjust the models to fit the data as closely as possible. Additionally, we employed spectral analysis (Hendy and Penny, 1993) to help assess the accuracy of the phylogeny and to distinguish among specific hypotheses regarding the taxa (Lento *et al.*, 1995). Finally, to statistically evaluate the likelihood of different colonization hypotheses, we recoded them as multistate characters and estimated their fit to the mtDNA phylogeny using randomization procedures.

MATERIALS AND METHODS

Subspecies and Genes Sequenced

To choose individuals to represent the subspecies of common chaffinches, a 600-bp segment at the 3'-end of the control region was sequenced for several individuals from representative populations of each subspecies (Fig. 1). Most subspecies were found to be characterized by a small number of very similar haplotypes, and the most common was chosen for the present study. Two distinct haplotypes were found in each of *F. c. canariensis* and *F. c. maderensis*, and representatives of both were selected here. The second *F. c. canariensis* haplotype was exclusive to one population (Gran Canaria). Genetic diversity within and among populations of Atlantic island common chaffinches is beyond the scope of this study and is the subject of a manuscript in preparation (Marshall and Baker, unpublished data). For the present study, we sequenced portions of four mitochondrial genes (cytochrome *b*, ATPase 6, NADH dehydrogenase subunit 5, and the control region) for two individuals of each representative haplotype. Details of the primers used, regions of the genes sequenced, and DNA extraction and sequencing methodology are described elsewhere (Marshall and Baker, 1997, 1998).

Phylogeny Estimation

In previous work (Baker and Marshall, 1997), using sequences of the 3'-end of the control region, we reconstructed the phylogenetic relationships among several haplotypes of common chaffinch, the blue chaffinch, the congeneric brambling (*F. montifringilla*), and a closely related cardueline finch, the greenfinch (*Carduelis chloris*). This study demonstrated that a haplotype found only in Nefza, which we identified as *F. c. spodiogenys*, is genetically intermediate between the blue chaffinch and other common chaffinch haplo-

types and is the sister taxon to the other common chaffinches. Therefore, to examine the relationships among common chaffinch subspecies in this study, we used this haplotype as the outgroup. In phylogenies derived from DNA sequence data, use of the most closely related outgroup is preferable, since it increases the signal-to-noise ratio and potentially improves the probability of accurately estimating the phylogeny.

Prior to phylogeny estimation, we assessed rate variation among lineages by calculating likelihood estimates for trees generated with and without the assumption of equal branch lengths, using the DNAMLK and DNAML routines in PHYLIP 3.5 (Felsenstein, 1993). A *G* test of goodness of fit indicated that rate variation among lineages was significant ($G = 18.4$, $df = 8$, $P = 0.015$). We also calculated estimates of the α and κ parameters by an iterative procedure that involved generating parsimony estimates of the values using the PAMP program in the PAML computer package (Yang, 1995) and a star tree, calculating a maximum likelihood tree and likelihood value using these estimates and the BASEML program of the same package, recalculating the parsimony estimates of the parameters with the resulting tree, and recalculating the maximum likelihood estimate with the new parameter estimates until the likelihood value remained constant. The α parameter denotes the shape of the γ distribution and as such is inversely related to the magnitude of rate variation among sites in the sequence (Yang and Kumar, 1996), while the κ parameter accounts for the transition to transversion (ts/tv) bias in the data. Phylogenetic relationships were assessed using neighbor joining, maximum likelihood, and parsimony methods, without the assumption of equal branch lengths. Initially, we performed phylogenetic analyses on the complete data set (including all sites) and attempted to evaluate different models of evolution (see below). We then repeated the analyses but excluded multistate characters from the data.

Neighbor joining. First, a neighbor-joining tree (Saitou and Nei, 1987) was constructed using Kimura 2-parameter distances (Kimura, 1980), as suggested by Kumar *et al.* (1993) when distances are small ($d < 0.5$) and ts/tv ratios high (ts/tv > 2). The computer program MEGA (Kumar *et al.*, 1993) was used, and bootstrap confidence levels (BCL; 500 replicates) and branch length confidence probabilities (CP) were calculated. Next, the rate variation parameter ($\alpha = 0.1138$) was incorporated into distance estimation. Branch length CP values are unavailable for this distance estimation method, but BCL values were generated as before. Third, we incorporated the ts/tv ratio parameter ($\kappa = 5.8351$) into distance estimation as implemented in PHYLIP 3.5 (DNADIST and NEIGHBOR programs; Felsenstein, 1993); no confidence testing was performed. Finally, we deleted multistate characters from the data set and repeated the first procedure.

Maximum likelihood. Maximum likelihood phylogeny estimation was executed using the DNAML program of PHYLIP 4.0 (Felsenstein, 1995). The κ -parameter estimate ($\kappa = 5.8351$) was incorporated. Additionally, six classes of rates were designated, three to account for each codon position in the coding genes and another three to accommodate the three different domains of variability in the control region (see Marshall and Baker, 1997). The relative rates, calculated by averaging pairwise Kimura 2-parameter distance estimates among all taxa for that class of sites, were 0.025, 0.013, 0.042, 0.027, 0.009, and 0.058, for first, second, and third codon positions, and Domains I, II, and III of the control region, respectively. The input order of taxa was randomized and global rearrangements were performed. The analysis was repeated 100 times on bootstrap replicate data sets (supplied by BOOTSTRAP in PHYLIP 4.0) and a majority-rule consensus tree was generated (using the CONSENSE routine in PHYLIP 4.0). Two such analyses were performed, one including all sites and the other excluding multistate sites.

Parsimony. Parsimony analyses were performed using PAUP 3.1 (Swofford, 1992). The exhaustive search option was used to obtain treelength frequency distribution skewness estimates (g_1), number and length of equally most parsimonious trees, and consistency indices (CIs). We then performed 1000 bootstrap replicates using the branch-and-bound option, from which a consensus tree (retaining all groups compatible with the 50% majority rule consensus tree) was derived. Characters were sampled equally, and weights were applied. Multistate characters were kept unordered throughout. Four weighting schemes were employed: (1) equal weighting of all characters, (2) transversion changes were given six times the weight of transitions using a symmetrical step matrix, (3) characters were weighted according to their relative divergence rates, as described above (weights of 2, 5, 1, 2, 6, and 1, were applied to first, second, and third codon positions, and Domains I, II, and III of the control region, respectively), and (4) binary transversion characters were given six times the weight of transversions and multistate characters using a step matrix.

Spectral Analysis

The properties and application of spectral analysis were described by Lento *et al.* (1995). Spectral analysis, as implemented in the HADTREE program (Hendy and Penny, 1993), employs the Hadamard conjugation (a discrete Fourier transform) together with the Cavender (1978) model of sequence evolution to correct the data for unobserved changes. Then, a phylogenetic spectrum is generated to represent the frequency of support for each split in the data set. A split is the separation of the taxa into two groups containing different nucleotides; a split occurs at each nucleotide site in the data set. Split frequencies (support values) are calculated by dividing

the sum of occurrences of that split by the total number of sites. In addition, the conflict for each split is quantified by summing all the splits that contradict the split in question. Split conflicts are normalized by multiplying them by the ratio of the sum of all support values to the sum of all conflict values, allowing both support and conflict to contribute equally to the assessment of a split. A choice of methods is offered for recoding multistate characters; we chose first to average the results from all methods (called "sum-of-7") and then to exclude the multistate characters. The closest tree method (a compatibility algorithm based on minimizing the sum of squares; Penny *et al.*, 1993) was used to select a tree depicting the splits that determine the phylogenetic relationships among the taxa.

Testing Alternative Colonization Routes

To determine the most likely route of colonization of the Atlantic islands by continental common chaffinches, we coded three possible routes as ordered multistate characters (colonization route characters; Fig. 2). Character 1 represents the possibility that birds went from Africa (*africana*) to the Canaries and separately from Iberia (*coelebs*) to the Azores, and character 2 represents the scenario whereby one wave of colonization occurred from Iberia to the Azores and subsequently to Madeira and the Canaries. As an alternative, we evaluated the possibility that there was one wave of colonization from the continent to the islands, but that it occurred via the Canaries through Madeira to the Azores (character 3). To code these scenarios as multistate characters, we first constructed the phylogenetic relationships which would be expected among the taxa if the postulated route had occurred, placed state changes at the nodes (but not at terminal taxa) and coded the taxa (starting with 0 for the basal node) according to the state at the most recent node. The expected relationships under each scenario were as follows: (1) [*spodiogenys* [*moreletti* [[*coelebs*, *africana*] [Canaries and Madeira haplotypes]]]], (2) [*spodiogenys* [[*coelebs*, *africana*] [*moreletti*] [Canaries and Madeira haplotypes]]]], and (3) [*spodiogenys* [[*coelebs*, *africana*] [Canaries haplotypes [*moreletti* and Madeira haplotypes]]]]. Each colonization route character was coded with the same number of states and steps on the shortest (expected) tree.

We then calculated the fit of each character on the mtDNA phylogeny. To assess the significance of the differences in fit among characters, we randomized each colonization route character 1000 times (using the multiply and shuffle options of MacClade 3.01; Maddison and Maddison, 1993), plotted the distribution of the fit of each randomized character on the mtDNA phylogeny, and found the probability of obtaining the fit of the actual character by chance alone. We then produced 10,000 equiprobable random trees from the taxa (using MacClade), plotted the distribution of the fit of each

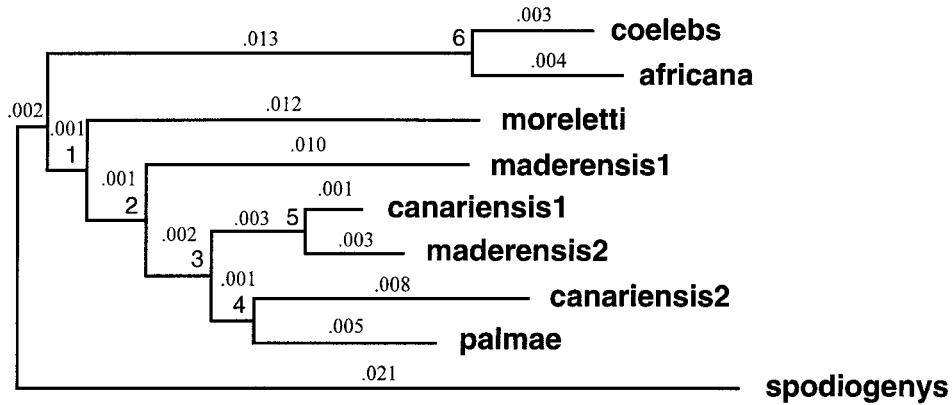


FIG. 3. The tree topology recovered from all neighbor-joining analyses. Numbers on the branches are branch lengths obtained using non-rate-adjusted distances and the complete data set. Bootstrap confidence levels and branch length confidence probabilities for each numbered node are in Table 1.

and patterns of evolution of these genes were described in detail previously (Marshall and Baker, 1998).

Initial Phylogenetic Analyses

The topologies resulting from the analyses involving the complete data are presented in Figs. 3 (neighbor joining), 4A (maximum likelihood) and 5A and 5B (parsimony), and several features are immediately apparent. First, the two continental haplotypes (*coelebs* and *africana*) always cluster together, as do one each of

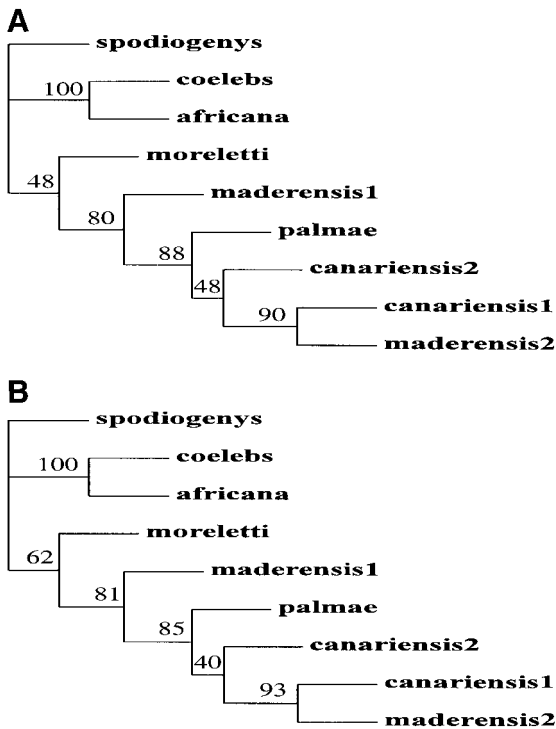


FIG. 4. Tree topology and bootstrap confidence levels obtained from maximum likelihood analyses. (A), the complete data set. (B), multistate characters excluded.

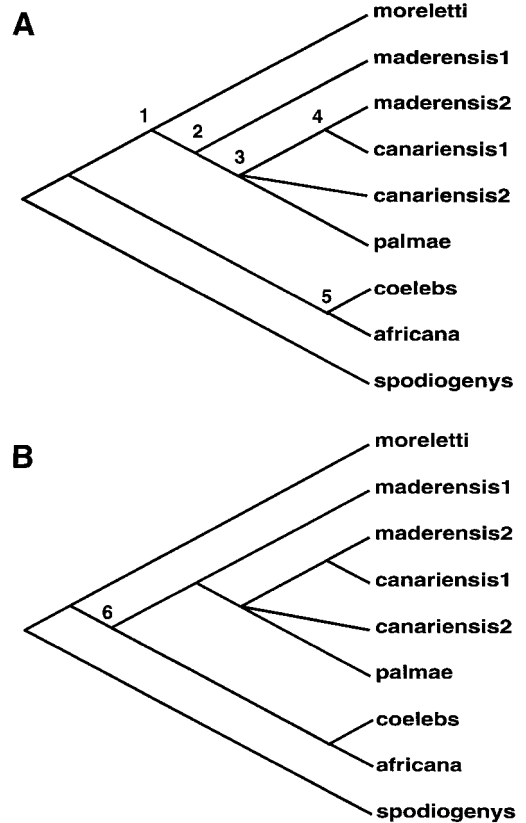


FIG. 5. Tree topologies obtained from parsimony analyses. (A), equal weighting, rates weighting, and transversion weighting with multistate characters excluded. (B), transversion weighting of the complete data using a step matrix. Both tree topologies represent bootstrap consensus trees, and bootstrap support at the numbered nodes is contained in Table 2 for each of the four weighting schemes.

the Canaries and Madeira haplotypes (*maderensis2* and *canariensis1*). Additionally, the clades clustering all of the Canaries and Madeira haplotypes, and all of the Canaries and Madeira haplotypes with the exception of *maderensis1*, are also conserved. The Azores haplotype (*moreletti*) usually occurs as the sister taxon to the other island haplotypes, but in the parsimony analysis involving transversion weighting it becomes the sister taxon to all other members of the ingroup. Similarly, the position of one Canaries haplotype (*canariensis2*) changes among analyses.

The associated bootstrap and branch length confidence probabilities (where applicable) at each node are found in Tables 1 (neighbor joining) and 2 (parsimony) and Fig. 4A (maximum likelihood). The continental clade (*coelebs*, *africana*) is always very highly supported by these statistics, regardless of method or model. Support for the other conserved nodes tends to be reasonably high (greater than 80), but varies, most notably among parsimony analyses. For instance, bootstrap support for the clade containing all Madeira and Canaries haplotypes decreases from 84 with transversion weighting to only 45 when sites are weighted according to substitution rate. On the other hand, when transversions are weighted the bootstrap value for the *maderensis2/canariensis1* cluster drops to 66 from more than 90 under equal or rates weighting. Not surprisingly, support values for the four variable clades are generally lower (under 80). In particular, using parsimony, the clade clustering all islands is supported by 68–74% of bootstrap replicates, although the contradictory clustering with the continental clade inside the island clade (polyphyly of the island clade) receives even poorer bootstrap support (62). Similarly, using parsimony, the position of *canariensis2* is equivocal (polytomous on strict consensus trees).

TABLE 1

Bootstrap Confidence Levels and Branch Length Confidence Probabilities Supporting the Phylogenetic Relationships Among Haplotypes of Common Chaffinch Obtained by the Neighbor-Joining Method

Phylogenetic relationships supported	Complete data		With α	Only binary sites	
	BCL	CP	BCL	BCL	CP
1 ^a All island haplotypes	72	73	56	85	89
2 All Canaries and Madeira haplotypes	86	91	76	69	78
3 ((<i>canariensis2</i> , <i>palmae</i>) (<i>maderensis2</i> , <i>canariensis1</i>))	87	93	77	87	94
4 <i>canariensis2</i> and <i>palmae</i>	78	66	74	61	37
5 <i>maderensis2</i> and <i>canariensis1</i>	95	99	88	93	99
6 <i>coelebs</i> and <i>africana</i>	100	99	100	100	99

^a Number refers to node in Fig. 3.

TABLE 2

Statistics Describing the Phylogenetic Relationships Among Haplotypes of Common Chaffinches Obtained Using Parsimony and Four Weighting Schemes

Phylogenetic relationships supported	Bootstrap support			
	Equal	Tv	Rates	Binary
1 ^a All island haplotypes	68	—	74	76
2 All Canaries and Madeira haplotypes	71	84	45	88
3 ((<i>canariensis2</i> , <i>palmae</i> , (<i>maderensis2</i> , <i>canariensis1</i>))	88	63	67	61
4 <i>maderensis2</i> and <i>canariensis1</i>	92	66	99	69
5 <i>coelebs</i> and <i>africana</i>	100	100	100	100
6 ((<i>maderensis1</i> , (<i>maderensis2</i> , <i>canariensis1</i>) <i>canariensis2</i> , <i>palmae</i>)) (<i>coelebs</i> , <i>africana</i>))	—	62	—	—
CI	0.66	NA	0.67	0.70
<i>g1</i>	-1.76	-2.03	-1.58	-1.92

^a Number refers to node in Fig. 5.

Tree Confidence, Spectral Analysis, and Tree Selection

Because bootstrap and branch length confidence probability values are moderate and tree topology volatile at certain nodes, the phylogenetic positions of two haplotypes (*moreletti* and *canariensis2*) are uncertain. In the case of *canariensis2*, no consensus is obvious and it seems best to treat the (*canariensis2*, *palmae*, (*canariensis1*, *maderensis2*)) cluster as a polytomy. With respect to the *moreletti* haplotype, polyphyly of the island clade occurs only once among six models. However, congruence in topology obtained from different methods does not constitute support for a particular tree, and an alternative approach is needed to assess tree reliability and to choose among models and resulting topologies. The polyphyletic topology has weaker bootstrap support than the clade uniting the island haplotypes, particularly in the parsimony model involving rates weighting. The *g1* estimate (Table 2), which describes the skewness of treelength distribution resulting from each data set, is lowest for the transversion weighting model, but indicates significant structure in all cases (Hillis and Huelsenbeck, 1992). The consistency indices of the parsimony trees (Table 2), although moderately high for molecular data, could not be used to choose among trees, as this statistic is not available for the transversion weighting model (which involved a step matrix). In an attempt to circumvent this, transversion weighting was applied site by site (that is, by weighting sites that contain a transversion 6:1). Surprisingly, however, this procedure returned the more common tree topology whereby the island haplo-

types form a monophyletic group, although with an improved CI (0.74).

Clearly, a dissection of the character support for these alternative groups is required, and spectral analysis provides one means to do this. The phylogenetic spectra for the two methods of handling multistate characters are presented in Fig. 6. Notably, the closest tree returned for each (inset of Fig. 6) is identical to the neighbor-joining result; that is, *moreletti* clusters with the other island haplotypes, and *canariensis2* clusters with *palmae*. The spectra provide the amount of support and conflict for each split, and we can examine how this relates to which splits are incorporated into the tree. Because there are nine taxa, the fully bifurcating tree will contain the 6 splits with the highest support minus conflict values that do not contradict better-supported splits already in the tree. Thus, for example, split 127 (which clusters *africana* and *spodiogenys*) is not in the tree despite its high support value because it also has a high conflict value. On the other hand, split 6 (*maderensis1* and *maderensis2*), which ranks quite highly in overall support minus conflict, is not in the

tree because it contradicts the higher ranking split 12 (*maderensis2* and *canariensis1*). Similarly, splits 224 and 3 are not included in the tree. The highest ranking split is 192, which clusters the continental haplotypes (*coelebs* and *africana*), followed by splits 12 and 60, which define the Madeira and Canaries groups found in previous analyses.

Of particular interest are splits 63, 254, and 48. Split 48 defines the *canariensis2/palmae* clade which was not unambiguously resolved in the previous analyses. Despite its relatively poor support minus conflict ranking it is included in the closest tree, presumably because it does not contradict other splits. Conversely, the contradictory split (containing *maderensis2*, *canariensis1*, *canariensis2*) found in the maximum likelihood and parsimony analyses, does not rank in the top 15 splits. Split 63 defines the controversial island monophyly clade and ranks fourth highest in both spectra. In the "sum-of-7" spectral analysis (Fig. 6A), the support for this split is only marginally better, and conflict only slightly lower, than for the contradictory split 254, which clusters *moreletti* with *spodiogenys* and makes the island taxa polyphyletic. This helps explain why these two groups are not easily resolved by bootstrapping. However, when multistate characters are removed from the analysis (Fig. 6B), support for split 254 decreases, while conflict for the 2 splits remains equal, resulting in a much lower ranking for split 254.

Because the elimination of multistate characters had such a dramatic impact on the ranking of different splits, the phylogenetic analyses described previously were repeated, but with the multistate characters excluded. With each method, this resulted in the monophyletic island topology (Figs. 3, 4B, and 5A) being recovered with substantially increased bootstrap and branch length confidence probability support. The multistate characters were then examined in detail by mapping their state changes onto the two contradictory trees (Fig. 7). There were four potentially phylogenetically informative multistate characters, three of which had equal numbers of steps on each tree (one transition and one transversion). A fourth (character CRI 245 in Fig. 2) also required two steps on each tree, but on the island monophyly tree it required two transversions whereas on the island polyphyly tree it required one transition and one transversion. This explains why transversion weighting using a step matrix resulted in the island polyphyly tree, while weighting transversions site by site (which also weights the transition changes at multistate sites) results in the island monophyly tree.

Thus a major factor causing the data to support the polyphyletic island tree is the weighting of a single multistate character in the control region according to transversion bias. This multistate site occurs in a region of the control region with a low α parameter describing the γ distribution of rate variation among

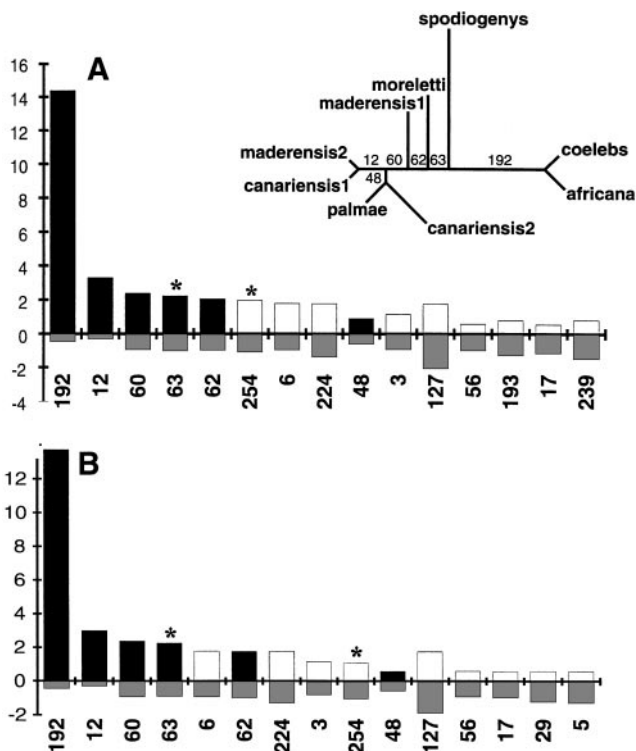


FIG. 6. Phylogenetic spectra. (A), sum-of-7 method of dealing with multistate characters. (B), multistate characters excluded. Above the axis is graphed support for the split, and below it, conflict. Splits are ranked according to overall support (support minus conflict). Split numbers represent the sum of the binary numbers for the taxa included in one of the two groups generated by the split. The binary number for the i th taxon is 2^{i-1} . The inset shows the closest tree. Splits included in the tree are in black, and the two starred splits are those defining island monophyly and polyphyly.

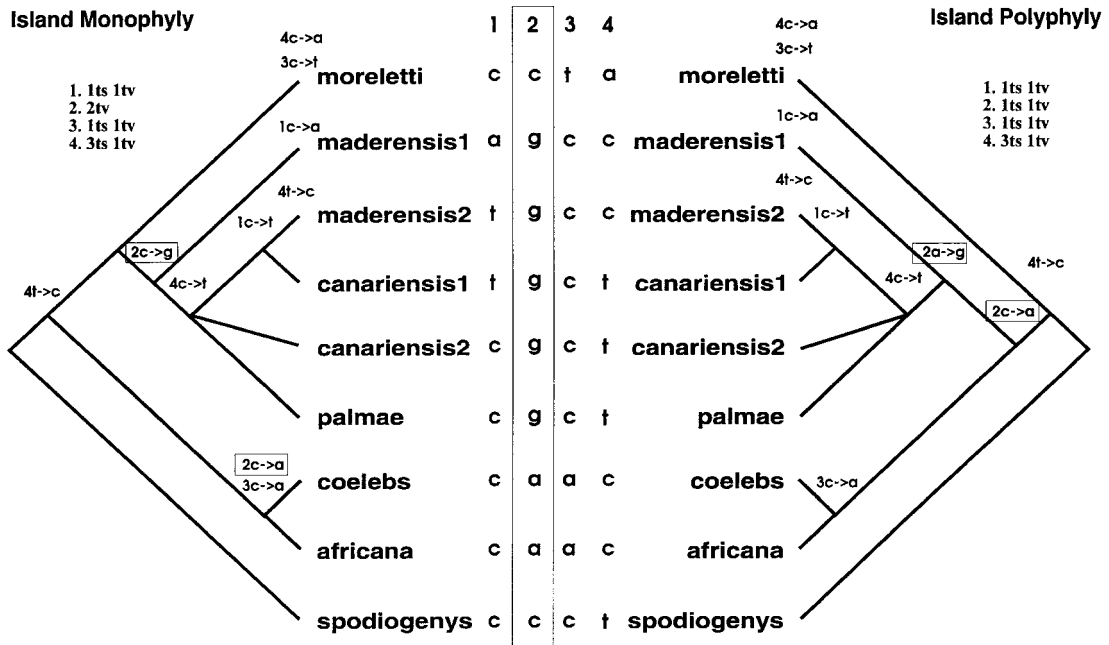


FIG. 7. Changes required in four phylogenetically informative multistate characters from the mtDNA data on the two possible tree topologies. Individual state changes are placed over the node where they occur, and text insets summarize the changes required. Tv refers to transversion and ts to transition. Boxes indicate the problematic character (see text).

sites ($\alpha = 0.4757$; Marshall and Baker, 1997). This means that this is likely a highly variant site (a “hotspot”) amid many invariant sites and is therefore subject to higher rates of evolution (evidenced by it having multiple states) and has a correspondingly higher chance of being homoplastic. Weighting the transversion change at this site thus seems questionable, if not inadvisable. Additionally, the higher bootstrap support for island monophyly versus polyphyly suggests that the greater balance of support is in favor of the former tree.

Distinguishing Among Colonization Route Hypotheses

On the “best tree” derived from phylogenetic analysis of the mtDNA data set (the island monophyly tree; Fig. 5A), the colonization route character with the best fit is character 2 (3 steps), the one describing a single wave of colonization from Iberia to the Azores, and subsequently to Madeira and the Canaries. Character 1, suggesting two waves from the continent separately to the Azores and the Canaries, is only one step longer, and the third possibility, a single wave but going first to the Canaries, is the longest (5 steps). Character 2 is significantly shorter on the best tree than would be expected among random characters ($P = 0.0030$); this probability is higher for character 1 ($P = 0.048$) and very high for character 3 ($P = 0.157$). Similarly, the fit of character 2 to the best tree is significantly shorter than the fit to a random tree ($P = 0.0031$, compared with $P = 0.036$ for character 1 and $P = 0.012$ for character 3). Finally, the mean fit of character 2 to the 1000

bootstrap trees is lower than that of characters 1 and 3 (3.71 vs 4.13 or 5.11). The difference in steps on each tree when comparing characters 2 and 1, and 2 and 3, would not be predicted by chance alone under a binomial distribution ($P < 0.00001$ in each case).

DISCUSSION

Confidence in the Molecular Phylogeny

The two major components of phylogeny inference from molecular data are choice of method and model for constructing a tree and assessment of confidence in the resulting tree. It has been suggested that, with respect to phylogeny reconstruction, “no current algorithm meets all the necessary criteria of being fast, efficient, consistent, robust, and falsifiable” (Penny *et al.*, 1992; Rohlf *et al.*, 1990). Furthermore, the model of evolution must be adjusted to fit the data as closely as possible, in the absence of actual knowledge of the processes of evolution responsible for the data. Because even randomly generated data can lead to a single best tree, once a topology has been constructed a statement of its reliability must be made (Hillis *et al.*, 1993). In particular, one must evaluate statistical support for different nodes in the tree and compare the tree to alternative well-supported topologies. Finally, congruence among independent data sets representing both molecular and nonmolecular information is the ultimate indicator of phylogenetic reliability (Hillis *et al.*, 1993).

To address these problems, we selected three popular

but philosophically different approaches to phylogeny reconstruction, one distance based, one character based, and one statistical. Within each method, we adjusted the model to incorporate parameters estimated from the data. Thus, with the neighbor-joining algorithm we compared default distances with rate-adjusted distances; using the parsimony approach we evaluated weighting schemes meant to account for apparent rates of evolution in different kinds of characters; and with maximum likelihood we adjusted the model to account for rate differences between transitions and transversions, and at different types of sites. To assess confidence in and choose among alternative topologies, we compared bootstrap confidence levels and branch length confidence probabilities at different nodes, as well as consistency indices and *gI* estimates from the parsimony analyses. We also examined phylogenetic spectra representing character support for different groupings of the taxa.

The result was that most relationships among common chaffinch taxa appear well defined, but the positions of two taxa are problematic. Most importantly, bootstrap and branch length confidence levels are somewhat weakly supportive of the clade defining island monophyly, although better than for the alternative polyphyletic grouping. Spectral analysis proved useful in revealing why the data lead to these somewhat ambiguous results and confirmed the presence of more support for the island monophyly hypothesis relative to the island polyphyly possibility. Subsequent inspection of multistate characters implicated transversion weighting of a single character as sufficient to upset the balance in favor of the polyphyly topology. Overall we feel reasonably confident that the data provide greater support for the island monophyly tree; however, the alternative island polyphyly hypothesis cannot be discarded. Supporting the island monophyly hypothesis, three independent data sets (song memes, morphometrics, and allozymes) return a monophyletic island clade upon cluster analysis (Lynch, 1991; see also Baker *et al.*, 1990, and Dennison and Baker, 1991).

These results provide two important cautions to investigators using DNA sequence data to resolve phylogenies. The first is the possible inadvisability of weighting transversions that occur at multistate sites. Because these sites may be highly variable they may be subject to homoplasy, and weighting would result in an incorrect topology or decreased confidence in the resulting tree. The second concerns the feasibility of resolving certain phylogenetic problems with sequence data. The problematic node in the common chaffinch phylogeny stems from a short branch occurring deep in the phylogeny. Using a rough calibration of the evolutionary rate of mitochondrial DNA for birds (2% per million years per lineage; Shields and Wilson, 1987) and the branch lengths provided by the neighbor-joining analyses, the branch leading to the island clade persisted for

about 50,000 years, while that leading to the present-day *moreletti* haplotype represents 600,000 years of evolution. Clearly, the phylogenetic signal defining the short island branch will be greatly obscured by the long period of evolution affecting subsequent lineages. Even with the relatively large data set presented here, confidence in this branch is not overwhelming and the node is sensitive to the weighting of a single character. With a much larger data set, this node may be more stable, but it is doubtful that bootstrap or branch length support would increase substantially.

Colonization of the Atlantic Islands by Chaffinches

On the better-supported island monophyly tree, the colonization route character with the fewest steps is consistent with one wave of colonization from the continent to the islands via the Azores. This best-fit character is significantly shorter on the mtDNA tree than a randomly shuffled character with the same states would be on this tree or than this character would be on a randomly generated tree. These observations make intuitive sense, given that the expected tree under this colonization scenario is very similar to the topology actually obtained. Additionally, this topology occurs significantly more often than would be predicted by chance among bootstrap replicates of the data. Support for the other two colonization route hypotheses is weak, and is particularly poor for the scenario involving a single wave of colonization via the Canaries rather than the Azores.

The implications of the mtDNA phylogeny regarding the colonization of the Atlantic islands are as follows. First, remnants of an ancestral lineage of common chaffinch still present in Nefza, Tunisia, gave rise to the ancestor of present day continental haplotypes. Then, in rapid succession (according to comparison of neighbor-joining branch lengths), this ancestor colonized the Azores, followed by Madeira and the Canaries. While a separate lineage may have occurred in Iberia at the time and provided the source population for the invasion of the islands, there is no evidence that it persists today (divergences between *coelebs* and *africana* haplotypes are very small compared with divergences of either from *spodiogenys*.) This is probably because, subsequent to the invasion of the islands, the older European populations were thought to be extirpated by Pleistocene glaciations and later recolonized from north Africa. Therefore, in hypotheses of colonization, Iberian haplotypes were not expected to cluster with Azores or island clades.

Within the islands, Madeira was likely originally colonized before the Canaries based on geographical proximity to the Azores, and because Madeiran haplotypes would otherwise occur only within the Canaries clade. However, the strong association of one Madeira haplotype with a Canaries haplotype indicates that subsequent back colonization from the Canaries to

Madeira must have occurred. Patterns of song meme diversity in the Madeiran populations also indicate admixture from both the Azores and the Canaries archipelagoes (Lynch and Baker, 1993). Routes of colonization within the islands of the Canaries archipelago are not obvious from the mtDNA phylogeny due to the uncertainty of relationships within the associated clade. One possibility, consistent with the maximum likelihood topology, is that birds arrived first in the western islands of La Palma and Hierro (represented by *palmae*) and went next to the central islands of Gomera and Tenerife (represented by *canariensis1*), and finally moved east to Gran Canaria (*canariensis2*). Short branch lengths combined with low bootstrap and branch length confidence levels within this clade suggest that, whatever the route, this was a rapid series of events.

A single wave of colonization seems more plausible from a variety of ecological and environmental perspectives as well. First, strong winds are thought to blow southeastwards from the Azores to the Canaries in winter when chaffinches are in flocks of substantial size (Fernadopullé, 1976; Grant, 1980). Indirect support is provided by another species of bird, the robin *Erithaca rubecula*. Separate Canary island populations of this species appear to have been established from both north Africa (to the eastern islands of Tenerife and Gran Canaria) and Europe (to the western islands of Gomera, La Palma, and Hierro), based on distinct plumage similarities to the different continental subspecies. The European source may have been the Azores, as Azores robins of European extraction share a distinctive pale plumage with the European-derived Canaries populations. Finally, island common chaffinches share plumage characteristics which set them apart from continental subspecies. In particular, their dorsal plumage is a slateblue colour, and they exhibit reduced amounts of green and red coloration compared with continental birds (Grant, 1980). As the islands are ecologically and climactically quite different from each other, it is difficult to reconcile these similarities entirely with convergent evolution in a common environment.

The major evolutionary conclusion of this study is therefore that patterns of similarity among Atlantic island common chaffinches are due to common colonization history rather than convergent evolution in a common island environment. That is, distinct island traits such as blue pigmentation, short wings, and long tarsi could have evolved only once on the Azores, rather than independently on the different islands. Furthermore, with respect to the unusual beak shape found in birds from the Canaries and Madeira, character displacement by the blue chaffinch is an unlikely explanation if Madeira was colonized before the Canaries because blue chaffinches are not found on Madeira and the narrower bill of Canaries chaffinches could have evolved there before Madeiran birds colonized the

Canaries. In summary, although the mtDNA phylogeny does not provide a full solution to the problems posed by Atlantic island common chaffinches, this approach to studying their evolution points to the value of phylogeny reconstruction for inferring colonization routes, and the importance of the colonization route for interpreting the evolution of island populations.

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