Rapid Radiation of Canaries (Genus *Serinus*)

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Mitochondrial cytochrome *b* (mit cyt *b*) DNA from 20 out of 37 extant canaries (genus *Serinus*) has been sequenced from living specimens photographed around the world. Phylogenetic analysis has consistently resulted in the same groupings of birds, which have generally been related to geographical proximity. The fossil registry of chicken and pheasant and its divergence time have been used to calibrate the molecular clock; mit cyt *b* DNA dendrograms suggest that the *Serinus* bird lineage appeared in the Miocene (9 MYA), a time when the Mediterranean Sea was closing its western and eastern oceanic connections. Pleistocene glaciations (starting 2 MYA) may have only been important in the subspeciation and isolation of birds in the Northern and Southern hemispheres around the world, and not only in North America, where it has already been described. The European-isolated *Serinus citrinella* (Citril finch) is not a canary but rather a true goldfinch. Only about 4% average nucleotide divergence is found among the different *Serinus* species; this suggests a remarkably rapid radiation when compared to other passerine (songbird) genera radiations. In addition, reproductive barriers are observed between closely related species but not between other more distant ones. Finally, a tentative classification for the genus *Serinus* species is put forward.

Introduction

Serinus (canaries) is a genus of finches belonging to the Fringillidae family of birds, which also includes many sparrows (genus *Passer*) and brambling and chaffinch. Most of them are beautifully colored and are widespread and familiar to bird watchers and other people (Armani 1983, pp. 21–131; Sibley and Monroe 1990, pp. 701–715; Clement, Harris, and Davies 1993, pp. 170–252). The relationships of the species within the genus *Serinus* and to other Carduelinae finches have not been fully resolved (Fehrer 1993; Fehrer 1996), particularly regarding Old World species (Marten and Johnson 1986). Parallel evolution of many characters seems to be an obstacle to phylogenetic resolution (for review, see Fehrer 1996).

It is also intriguing that the *Serinus* species are mostly confined to Africa and the Mediterranean Basin; however, it is believed that their ancestors came from the Palearctic region (probably from somewhere around the Europe/Asia junctions [Clement, Harris, and Davies 1993, pp. 170–252]). Notwithstanding, two species, *S. thibetanus*, which exists in Central Asia, and *S. estherae*, which lives in parts of the Malay Archipelago, are relict species isolated from the Mediterranean-African area (Clement, Harris, and Davies 1993, pp. 170–252).

Although many scholars think that Pleistocene temperature variations (glaciations) and the subsequent isolation are the most important factors that provoke the appearance of new extant bird species (Brooke and Birkhead 1991, pp. 79–86; Gill 1995, pp. 39–43 and 130–131), recent contradictory evidence exists which suggests that speciation of some genera and orders may have occurred long before (Chiappe 1995; Feduccia 1995; Hackett 1996; Hedges et al. 1996; Härlid, Janke, and Arnason 1997), particularly in passerines (Klicka and Zink 1997) and in Carduelinae (Marten and Johnson 1986; Fehrer 1996). In the present work, we have collected 20 *Serinus* species samples from around the world in order to sequence an orthologous gene from each species: the mit cyt *b* (924 bp). Mitochondrial DNA has proven to be helpful for defining the evolutionary relationships among relatively distantly and closely related birds and other species (Arnason and Gullberg 1994; Seutin et al. 1994). We have also aimed to study the relatedness of these bird species in the context of the paleogeography and the molecular clock timing in order to get an overall picture of the phylogeny and time of appearance of extant *Serinus* species (Paturi 1991, pp. 284–496; Smith, Smith, and Funnel 1994, pp. 24–39; Cox and Moore 1995, pp. 134–276). Furthermore, if this particular task is achieved, more precise data could be added to clarify the present controversy about more recent versus older (Klicka and Zink 1997) radiation of passerine birds.

Materials and Methods

Bird samples come from species and places that are detailed in table 1; GenBank sequence accession numbers are also given. Photographs were taken with a Nikon N-90 camera equipped with a Nikon 80-200 zoom lens and automatic flash. Blood from living birds (one individual per each species) was drawn after their claws were locally anesthetized with a lidocaine ointment and then cut. Blood was collected in EDTA cooled at 4°C and frozen until use. DNA was obtained and mit cyt *b* (924 DNA bases) was amplified with primers L14841 5′-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3′ and H15767 5′-ATGAAGGGATGTTCTACTGGTTG-3′, as detailed by Edwards, Arctander, and Wilson (1991). Polymerase chain reaction (PCR), cloning, and automatic DNA sequencing were performed as previously described (Edwards, Arctander, and Wilson 1991; Arnaiz-Villena et al. 1992). At least three clones from two different PCRs were sequenced from each species in order to assess sequencing quality. In the case of

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Table 1
List of Species, Origin, and Mit Cytochrome *b* Sequence Identification

Species	Mit cyt *b* Sequence	Sample Region
Canary (*Serinus canaria*)	L76266	Gran Canaria. Canary Islands, Spain
Canary (cagebird) (*S. canaria*, "Spanish" type)	L76277	Madrid, Spain ♀
Serin (*S. serinus*)	L76263	Madrid, Spain
Black-headed canary (*S. alario alario*)	L76276	Capetown, South Africa
Yellow-crowned canary (*S. canicollis flavivertex*)	L76295	Nairobi, Kenia
Yellow-crowned canary (*S. canicollis canicollis*)	L78706	Capetown, South Africa
Red-fronted serin (*S. pusillus*)	L77873	SinWiang, China
Yellow-rumped seedeater (*S. atrogularis atrogularis*)	L76267	Capetown, South Africa
White-rumped seedeater (*S. leucopygius riggenbachi*)	L76264	Dakar, Senegal
Lemon-breasted canary (*S. citrinipectus*)	L78707	Maputo, Mozambique ♀
White-bellied canary (*S. dorsostriatus dorsostriatus*)	L76278	Dar es Salam, Tanzania
African citril (*S. citrinelloides citrinelloides*)	L77555	Nairobi, Kenya
Yellow-fronted canary (*S. mozambicus mozambicus*)	L76265	Dar es Salam, Tanzania
Yellow canary (*S. flaviventris quintoni*)	L76280	Capetown, South Africa
Brimstone canary (*S. sulphuratus sulphuratus*)	L76294	Capetown, South Africa
Streaky-headed seedeater (*S. gularis endemion*)	L77556	Capetown, South Africa £
White-throated canary (*S. albogularis albogularis*)	L78705	Capetown, South Africa
Streaky seedeater (*S. striolatus striolatus*)	L77557	Nairobi, Kenya £
Tibetan siskin (*S. thibetanus*)	L76279	Szechwan, China
Chaffinch (*Fringilla coelebs coelebs*)	L76609	Madrid, Spain
Sudan golden sparrow (*Passer luteus*)	L76714	Dakar, Senegal

NOTE.—All of the specimens studied are males, with the exception of those marked with £ (undetermined sex) and with ♀ (females).

S. citrinella, samples from four different individuals were used since this bird should be reclassified within the genus *Carduelis* (goldfinches) according to the results herein obtained and according to Arnaiz-Villena et al. (1998). The power of mit cyt *b* (or other orthologous genes) DNA sequences for solving taxonomy problems may be fully shown by analyzing as many of the closest extant species as possible, as occurs in the present work. Three different phylogenetic tree–constructing methodologies were used in order to independently confirm the robustness of the topologies (Härlid, Janke, and Arnason 1997): unweighted parsimony, neighbor joining (NJ), and unweighted pair group with arithmetic mean (UPGMA). The matrix of genetic distances for the NJ tree was obtained by the maximum-likelihood method, and Kimura two-parameter distances were used for the UPGMA dendrogram; the UPGMA tree was also obtained for estimating coalescence times from known outgroups' divergence times (Vincek et al. 1997). Times of species divergence are only a rough estimate, particularly in our study, since only pheasant–chicken fossil record divergence timing is available. Thus, the time scale for the UPGMA tree was obtained by comparing mit cyt *b* of chickens (Desjardins and Morais 1990) and pheasants (Kornegay et al. 1993), two species that diverged 20–19 MYA (Helm-Bychowski and Wilson 1986). The comparison yields an evolutionary rate (per lineage) of 0.62×10^{-9} nonsynonymous substitutions per nonsynonymous site per year and 1.7×10^{-8} synonymous substitutions per synonymous site per year, so that the overall rate is $3.97 \times 10^{-9} \pm 0.37$ (Vincek et al. 1997). The standard error of the pheasant–chicken distance indicates that the calibration error is about 10%. Bootstrap values are calculated to test the topology robustness of trees (Felsenstein 1985), and low–bootstrap value branches are shown because the same tree branch is obtained by at least two different tree construction methodologies (Edwards 1995). Also, the number of

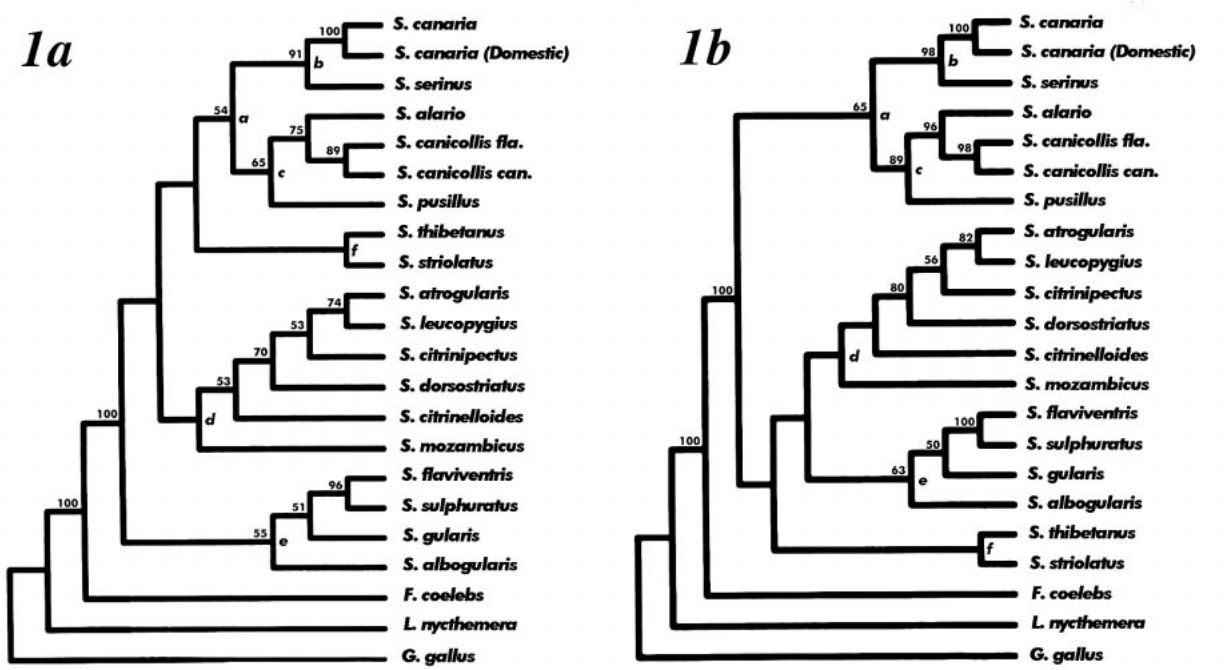

FIG. 1.—*a*, Unweighted maximum-parsimony heuristic search was used (PAUP), and 14 trees that were equally parsimonious were obtained. Consistency and retention indexes were 0.6 and 0.53, respectively. A majority rule bootstrap consensus tree based on 924 bases of mit cyt *b* genes from 20 *Serinus* species is shown. The transition/transversion ratio used was 2:1; the observed 5:1 ratio made no difference. Parsimony bootstrap analysis was done with 1,000 replications, and the values above 50 are shown. Parsimony was used unweighted (first, second, or third nucleotide) because weighting is recommended for higher evolutionary rates that were not expected in the relatively close species analyzed (Hillis, Huelsenbech, and Cunningham 1994). Mit cyt *b* from chaffinch (*Fringilla coelebs*), Pheasant (*Lophura nycthemera*), and domestic chicken (*Gallus gallus*) were also used as outgroups (Desjardins and Morais 1990; Kornegay et al. 1993). Letters a, b, c, d, e, and f point out the most significant nodes which are commented on in the text. *can. = canicollis*; *fla. = flavivertex. b*, Neighbor-joining bootstrap tree (1,000 replications) based on 924 bases of mit cyt *b* genes from 20 *Serinus* species. Bootstrap values above 50 are shown. The evolutionary model used was the minimum-evolution model. The transition/transversion ratio used was 2:1. Distance matrices were calculated based on maximum-likelihood analysis (Swofford 1996). Letters a, b, c, d, e, and f point out the most significant nodes which are commented in the text. *can. = canicollis*; *fla. = flavivertex.*

variable and phylogenetically informative sites (332 and 219, respectively, out of 924 mit cyt *b* DNA bases) is appropriate to establish sound phylogenetic comparisons (Hillis, Huelsenbech, and Cunningham 1994). After pilot studies, trees from *Serinus* and *Carduelis* (Arnaiz-Villena et al. 1998) birds were studied separately to achieve a more discriminating picture (De Queiroz, Donoghue, and Kim 1995), since both genera tended to establish separate groups within dendrograms, with the exception of the *S. citrinella* that was grouped in all trees obtained with the true goldfinches (*C. carduelis)* and thus should be reclassified as such. Genus *Carduelis* and genus *Serinus* do not join in phylogenetic trees to form a monophyletic group (not shown and Arnaiz-Villena et al. 1998).

Phylogenetic Analysis

The phylogenetic analyses were based on maximum parsimony, neighbor joining, and UPGMA (figs. 1 and 2). Subsequent analyses were designed to take into account levels of saturation (multiple substitutions at single sites) in different partitions of the data sets. Scatter plots were drawn that compared pairwise percent sequence divergence to pairwise transversion and pairwise transition divergences at first, second, and third codon positions. Two different estimates of percent divergence were used that serve as approximations of time since divergence: Kimura's (1980) two-parameter genetic distance and uncorrected pairwise divergence ($p = N_d/n$, where p is the percent sequence divergence, N_d is the number of nucleotides that differ between two sequences, and n is the total number of nucleotides compared; Nei 1987, Hackett 1996). Comparisons to sequences of the domestic chicken (*Gallus gallus*; Desjardins and Morais 1990) were used as a distant outgroup; the Sudan golden sparrow (*Passer luteus*) was also used as an outgroup that was theoretically closer than the chicken one.

Results and Discussion

Saturation is considered to have occurred in any of the data partitions if the scatter of points shows a leveling off of change as sequence divergence increases. Saturation plots for cyt *b* DNA (fig. 3) indicate that only third-position transitions show a clear leveling-off associated with saturation; this occurs through a 10% uncorrected sequence divergence. The method used for measuring genetic distance does not affect assessment of saturation since the use of Kimura's two-parameter distance shows the same saturation of the third position at 10% sequence divergence. None of the other data partitions (first-position transitions and transversions,

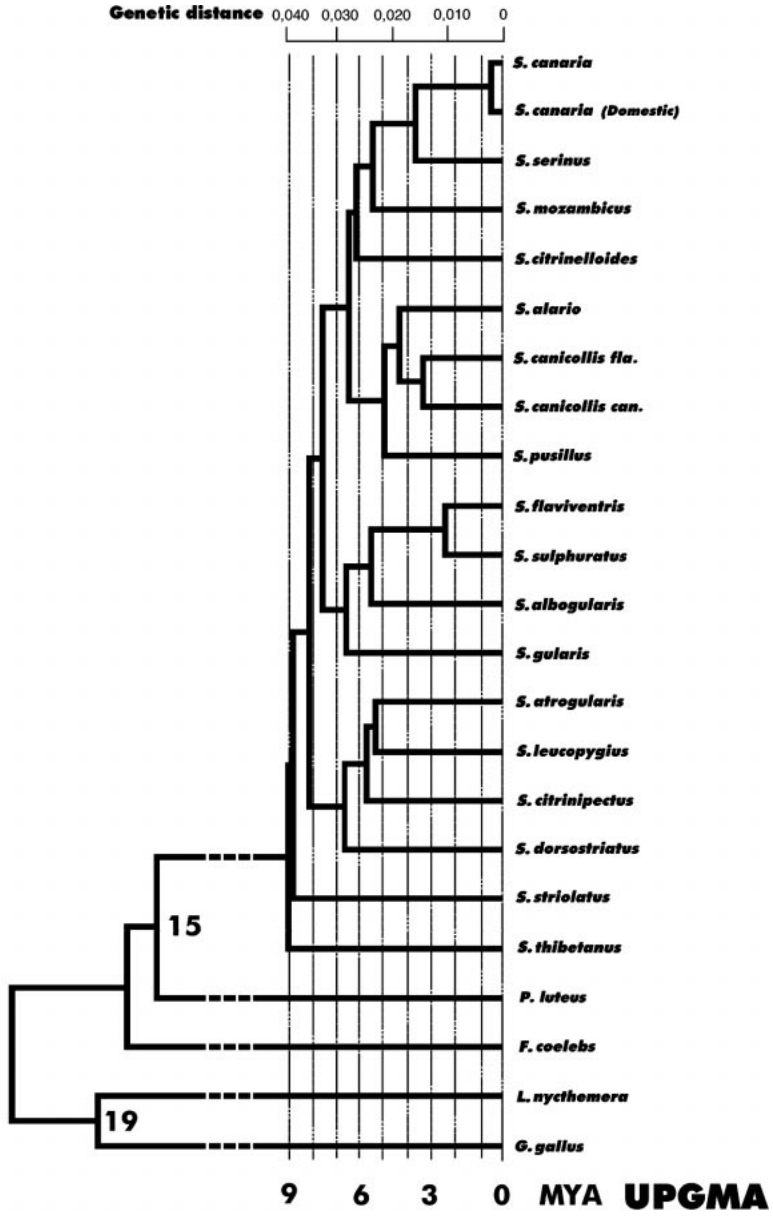

FIG. 2.—Approximate calculations on the time of appearance of genus *Serinus* lineages based on the known chicken–pheasant divergence time. Times were calculated from the known divergence time of pheasants (*Lophura nycthemera*) and chickens (*Gallus gallus*) and from their respective mit cyt *b* substitution rate comparisons (see Vincek et al. 1997). Dendrograms based on the unweighted pair group with arithmetic mean procedure and their corresponding Kimura biparametric distance matrices were obtained with the PAUP (version 4d50) and PHYLIP (version 3.5c) packages, kindly provided by D. L. Swofford (1996) and J. Felsenstein (1995), respectively. Unweighted pair group with arithmetic mean methodology tends to perform poorly if the assumption of equal rate of evolution among species (mit cyt *b*) is not respected. However, it seems to perform correctly in the closely related bird species used for this work since the groups of taxa are similar to those obtained in NJ and parsimony dendrograms (see fig. 1). The bird species and subspecies used are detailed in table 1; also, see footnote to fig. 1. Tree was rooted by *L. nychtemera* and *G. gallus* (Galliformes); also, *Passer luteus* (Passeriformes) has been added.

second-position transitions and transversions, and third-position transversions) show evidence of leveling-off and thus of saturation (fig. 3). Therefore, step-matrices were constructed in PAUP that down-weighted transitions over transversions by factors of 2 and 5 (see fig. 1 footnote) in order to take into account the observed saturation at third-position transitions.

The nucleotide distribution pattern of the mit cyt *b* gene of the *Serinus* birds under study was similar to the pattern found during previous analyses of this gene in birds and mammals (Kornegay et al. 1993). At the first codon positions, the four bases were equally distributed; at the second position, fewer G residues and a higher amount of T were seen. At the most variable third codon position, the bias against G and T was strong, as previously found by others (Edwards, Arctander, and Wilson 1991; Kornegay et al. 1993). This bias in base composition was similar in all species studied; however, *P. luteus* showed a higher G percentage (not shown), but it performed equally well as an outgroup when calcu-

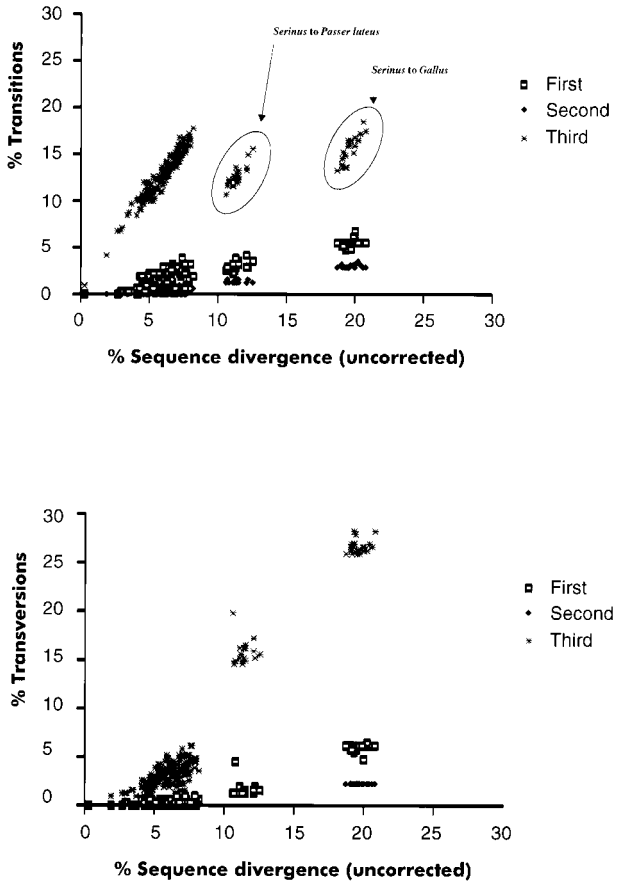

FIG. 3.—Saturation plots for the cytochrome *b* gene that relate uncorrected sequence divergence to changes due to transitions (top) and transversions (bottom) at first, second, and third codon positions.

lating parsimony, NJ, and UPGMA, since its sequence did not change the topology of the phylogenetic trees (fig. 2 and not shown). It has been suggested that standard parsimony methods can be unreliable when base composition varies (Lockhart et al. 1994). These authors suggested constructing phylogenetic relationships based solely on the observed nucleotide ratios among taxa. In our study, however, it seems that both *G. gallus* and *P. luteus* perform equally well as outgroups (figs. 1–3).

Cytochrome *b* gene variability was sufficient to establish phylogenetic relationships; most of the differences were silent substitutions, as is expected for a protein-coding gene, particularly for close relatives, such as species within a genus (Kocher et al. 1989). Within-species variability of the mit cyt *b* sequence was very low in the species tested (unpublished: *S. canicollis, S. canaria* (wild and cage), *S. striolatus, S. mozambicus, S. leucopygius,* and *S. gularis*). Our data are concordant with those already published (around 0.3% divergence, Avise and Ball 1991). Therefore, within-species variability was not likely to interfere with interspecific comparisons, which were in a higher range (see fig. 2).

As expected for a gene evolving rapidly under strong functional constraints, most (56%) of the third-position codons among *Serinus* species, in which substitutions are often silent, were variable. By contrast, relatively few of the first and second positions, 8.4% and 1.6%, respectively, were variable. Therefore, more than three-quarters (87.5%) of the phylogenetically informative sites occurred in third positions of codons. The slow accumulation of mutations in first and second positions of codons relative to third positions is further evident in the matrix of observed differences among chosen canary species (belonging to different branch groups; figs. 1 and 2), Sudan golden sparrow, chaffinch, and chicken (table 2).

Serinus Phylogeography and Tempo of Evolution

In order to estimate the tempo of evolution of *Serinus* species, the calculations done by Takahata, Grant, and Klein (Vincek et al. 1997) to assess the time of appearance of Galapagos Darwin's finches were followed. A UPGMA dendrogram (fig. 2) was constructed because this type of phylogenetic tree is more suitable for estimating coalescence time than other methods (Nei 1987). Next, a time scale for the UPGMA tree was obtained by comparing the cytochrome *b* DNA sequences of the pheasant (Wittzell et al. 1994) and the chicken (Zoorob et al. 1990), two species that are believed to have diverged around 19 MYA (Helm-Bychowski and Wilson 1986). The per-lineage comparison yields an overall evolutionary rate of $3.97 \times 10^{-9} \pm 0.37$. This

Table 2
Matrix of Observed Substitutions for First and Second Positions (Below Diagonal) and Third Position (Above Diagonal) in Cytochrome *b* Codons from Six *Serinus,* one *Fringilla,* one *Passer,* and *Gallus gallus* (See Table 1 for Scientific Names)

	Observed Substitutions with								
Species[a]	1	2	3	4	5	6	7	8	9
Canary (1)		38	56	49	36	57	90	86	129
Red-fronted serin (2)	4		51	58	43	50	89	87	125
Tibetan siskin (3)	10	10		60	52	64	101	91	139
White-rumped seedeater (4)	8	10	11		47	66	99	87	136
Yellow-fronted canary (5)	4	6	10	9		51	97	83	130
Streaky-headed seedeater (6)	7	7	9	10	7		95	93	131
Chaffinch (7)	17	17	17	21	21	20		99	123
Sudan golden sparrow (8)	17	19	21	20	17	20	26		133
Chicken (9)	50	51	52	53	52	51	51	52	

[a] Numbers in parentheses after species names correspond to column numbers to right.

substitution rate is approximately 0.4% per Myr, which roughly approximates the 4% of nucleotide substitution per lineage found between the most distant *Serinus* species, which arose about 9–10 MYA. A theoretical substitution rate of 2% per million years (Klicka and Zink, 1997) would result in a 20% average amount of nucleotide substitution (fig. 2). It is remarkable that the average percent of nucleotide divergence obtained among the 20 *Serinus* species is only 4% (see fig. 2); this value represents a surprisingly fast radiation for *Serinus* species (also suggested for Carduelinae by Fehrer, 1996). Other documented songbird radiations include the following: genus *Zonotrichia* has a 4.1% rate of nucleotide substitution, but for only seven species, and genus *Pipilo* has a substitution rate of 6.4% for only six species (Zink, Dittman, and Rootes 1991; Zink and Dittman, 1991). This molecular clock calibration may be correct because first, fossil data is included in the estimation of the species divergence time; this was considered accurate by Takahata, Klein, and Grant (Vincek et al. 1997). It is a clear advantage since the bird fossil record is fragmentary, particularly for small birds like *Serinus.* In the second place, the use of chicken and pheasant as outgroups seems to be correct, because saturation plots indicate that only third-position transitions appear to be saturated when both chickens and Sudan golden sparrows are tested as outgroups (fig. 3). However, the calculations of the times of species divergence need to be confirmed by other methodologies in this particular study and in others (i.e., Härlid, Janke, and Arnason 1997). In the latter study, passerines are found to be older than paleognathous birds; thus, the molecular clock calculations regarding bird evolution are still under debate. Thirdly, the Carduelinae species divergence timing that is described in the present study has also been suggested by others (Marten and Johnson 1986, Fehrer 1996). In particular, Fehrer (1996) finds that mit cyt *b* sequences are closer within Carduelinae groups than within other passerine groups. However, extensive microevolutionary studies that include many Carduelinae species within one genus were not available for further comparisons; the present study may help to understand better the suggested rapid evolution in Carduelinae. Nonetheless, the causes of the rapid radiation of *Serinus* (and of *Carduelis*; Arnaiz-Villena et al. 1998) need to be investigated further.

Although different *Serinus* groups are in general concordantly placed in dendrograms obtained by using parsimony and NJ (fig. 1) and UPGMA (fig. 2), some bootstrap values for nodes joining a few species belonging to otherwise well-established (high bootstrap) groups are low; this may mean that not all extant species are tested. This may not be the case for the *Serinus* species, since only species phenotypically related and geographically close to the tested ones are missing from the study (Sibley and Monroe 1990, pp. 701–715; Clement, Harris, and Davies 1993, pp. 170–252). In addition, parental species are extinct and/or each group of bootstrap-supported nodes represents radiations of separate subfamilies; furthermore, the species (and thus DNA) are too similar and have appeared within a relatively short time span. The latter two may be more favored hypotheses, and although more studies are necessary to support them, other close Fringillidae genera are definitively outgroups (these include several that already existed in the early Miocene epoch: *Passer, Lagnosticta, Lonchura, Pyrrhula, Rhodopechys,* and *Carpodacus*; Arnaiz-Villena et al. unpublished data). In general, established clades within dendrograms are geographically related.

Both parsimony and NJ trees (fig. 1*a* and *b*) basically established the same groups for genus *Serinus.* Node a groups canaries from the Mediterranean area (node b) and certain canaries from South Africa, Central Africa, and Asia (node c, fig. 4*a* and *b*) together. Canaries from the Mediterranean area comprise two species: serin and the wild canary from the Canary Islands; the caged domestic canary was bred in captivity for the first time about 500 years ago (when Europeans invaded the Islands) and was thus separated from the wild species. Also, the closest living relative to the wild canary is the Mediterranean serin, confirming the expectations (Sibley and Monroe 1990, pp. 701–715; Clement, Harris, and Davies 1993, pp.170–252) based on phenotypes (fig.4*a*), on fertile male hybrids and 20% female hybrids, and on geography. These Mediterranean canaries are probably linked to certain African and Asian *Serinus* birds by common and extinct ancestors (node c, fig. 1*a* and *b*). They are strikingly dissimilar in color and general phenotype. This may be a reflection on how climate changes during the last 7 Myr, when the ancestors of this group may have already existed (see below and fig. 2), and a relative isolation thereafter have caused drastic changes in color and bill shape (Gill 1995, pp. 39–43 and 130–131). Otherwise, random drift would be another explanation to the phenotypic changes observed. The oldest species is probably *S. pusillus* (Asian) (fig. 2), and this group (and the whole genus *Serinus*; Clement, Harris, and Davies 1993, pp.170–252) may be Asiatic in origin. In particular, the very recent Sahara desert desiccation which occurred about 10,000 years ago (Kutzbach et al. 1996) may have helped to separate the original Northwestern and Northeastern African members of the group under node a.

Node d (fig. 1*a* and *b*) groups together small African canaries (10–13 cm) which have a varied, powerful, and very nice singing, particularly the small and modestly gray-colored *S. leucopygia* (African singer; fig. 5*a*). This contrasts with other big African canaries grouped in node e (15–16 cm; figs.1*a*, 1*b*, and 5*b*), whose singing is more monotonous and not so varied and powerful. Big African canaries are all present in the extreme south of Africa (and also in Central Africa areas), while the small ones are more concentrated around the tropics; these size differences according to latitude are found in many species (Gill 1995, pp. 39–43 and 130–131). It is doubtful that big and small African *Serinus* finches have shared very close ancestors; if this is not so, the low bootstrap values linking small and big canaries may only reflect the lack of analysis of certain extant or extinct species. Relatedness of big and small African canaries with Mediterranean canaries may be

FIG. 4.—*a*, Mediterranean canaries: wild canary, endemic to the Canary Islands and also to the Azores and Madeira Islands (left); yellow domestic canary (right); and serin (above). They are grouped under node b in dendrograms 1*a* and *b*. The gray area is the distribution range for the represented birds. *b*, African–Asiatic canaries: these are grouped under node c with strong bootstraps (fig. 1) that raise little doubt about their relatedness in spite of evident phenotypic differences. Their habitats are fragmented, and ancestors (living 7 MYA, fig. 2) may have once occupied spaces between South Africa and China. Black-headed canary (left), yellow-crowned canary, nominal (below right), yellow-crowned canary, *flavivertex* (above left), and red-fronted serin (above right). The gray area is the distribution range.

FIG. 5.—*a*, Small African canaries are grouped under node d; however, the yellow-fronted canary and African citril may be also related to Mediterranean canaries (see bootstrap values in figs. 1*a*, 1*b*, and 2). Counterclockwise from upper left are shown the following: white-

relatively distant, since male hybrids obtained by crossing *S. canaria* with *S. sulphuratus*, *S. atrogularis*, and *S. mozambicus* are sterile (Baseggio 1995, pp. 116–198). However, male hybrid sterility may not be a sign of unrelatedness since F1 males from *S. canaria* and some South American siskins (i.e., *C. cucullata* and *C. xanthogaster*) are fertile (Baseggio 1995, pp. 116–198). This suggests that geographically closer species may develop hybridization barriers in the speciation process at meiotic, gamete, maturation, or other levels. The UPGMA dendrogram (fig. 2) further splits small African canaries and places *S. citrinelloides* and *S. mozambicus* with the Mediterranean canaries (they did not show high bootstrap values in parsimony and/or NJ trees); thus, a link between the two latter species may not be discarded even if sterile F1 males are obtained from *S. canaria* × *S. mozambicus* crosses (Baseggio 1995, pp. 116–198). In addition, the time of appearance calculation for African big canary species (fig. 2) supports the hypothesis that vertebrate speciation also took place before Pleistocene glaciations in the American (Arnaiz-Villena et al. 1998) and African Southern hemispheres, not only in North America (Klicka and Zink 1997).

Node f (fig. 1*a* and *b*) puts together two species that are very different in size (*S. striolatus*, 15 cm; *S. thibetanus*, 11 cm) and plumage (fig. 6); they are not related taxa according to the low bootstrap values obtained (see also UPGMA tree, fig. 2). They may be the most ancient extant canaries (see fig. 2) and may also be relicts of primeval canaries. Both lineages (and, in this case, species) seem to come from 9 MYA (Miocene, see fig. 2). *Serinus striolatus* may have become confined to Africa from a wider and also Asiatic range after climate changes occurred in the Middle East because of the Red Sea opening (10–5 MYA) and/or because of more recent glaciations (Paturi 1991, pp. 284–496). *Serinus thibetanus* may have become isolated after Himalayan and Alpine orogenesis (5 MYA). Both of them, according to our molecular data (Arnaiz-Villena et al. 1998), are included within the genus *Serinus* and not within *Carduelis*, as some authors have proposed (Clement, Harris, and Davies 1993, pp.170–252), and show that Miocene speciation also occurred in Eurasia.

In conclusion, phylogeny dendrograms of most *Carduelis* (Arnaiz-Villena et al. 1998) and *Serinus* genera extant species show that both radiations are intermingled in time but that both genera are not monophyletic; the earliest *Serinus* species appeared in the Miocene epoch about 9 MYA, slightly after the first *Carduelis* species (about 9.5 MYA), possibly in the Asian continent and coinciding with dramatic changes of the climate when the eastern Mediterranean Sea (Tethis Sea) was closing (Paturi 1991, pp. 284–496; Smith, Smith, and Funnel 1994, pp. 24–39; Cox and Moore 1995, pp.134–276). Other genera among the Carduelinae subfamily seem to be older (i.e., chaffinch, *Fringilla coelebs* [Marshall and Baker 1997], or its ancestors might have been present on Earth about 17 MYA in the early Miocene epoch; see fig. 2). Some species belonging to *Passer, Lagnosticta, Sicalis, Rhodopechis, Carpodacus* and *Pyrrhula* also seem to be earlier genera (early Miocene, A. Arnaiz-Villena, unpublished) and dendrograms place these latter finches as outgroups with respect to both *Carduelis* and *Serinus* species; this is in accordance with the published results of others about some North American passerines for whom alloprotein data were used (Marten and Johnson 1986). Thus, speciation timing of *Carduelis* (Arnaiz-Villena et al. 1998) and *Serinus* support the theories that some extant genera of songbirds were already present (or their ancestors were) before the Pleistocene (Klicka and Zink 1997); Pleistocene glaciations may have further driven speciation but on a scale lower than previously thought (Gill 1995, pp. 39–43 and 130–131).

Finally, the following groups of birds within the genus *Serinus* may be recognized when our molecular phylogenetic data as well as geographical data and the most evident gross phenotypic data (i.e., body size) are considered. Big and small African canaries had already been distinguished as different clades by using phenotype characters (van den Elzen and Nemeschkal 1991; Fehrer 1996). The grouping within genus *Serinus* may be as follows (distribution range shown in photographs has been taken from Clement, Harris, and Davies 1993, pp. 170–252): (1) Mediterranean canaries (fig. 4*a*); (2) Asian–African medium-sized canaries (fig. 4*b*), including the black-headed canary, yellow-crowned canary, and red-fronted serin (possibly related to Mediterranean canaries and once sharing or connected by Saharan habitat), which now exist in fragmented habitats (juvenile red-fronted serins [Asia] and black-headed canaries [South Africa] are almost identical [A. Arnaiz-Villena, personal observation]); (3) small African canaries (fig. 5*a*); (4) big African canaries (fig. 4*b*); and (5) relict canaries (fig. 6).

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←

rumped seedeater, yellow-rumped seedeater, lemon-breasted canary (female), yellow-fronted canary, white-bellied canary, and African citril. The gray area is the distribution range.

b, Large African canaries are grouped under node e (fig. 1*a* and *b*); they probably appeared in the extreme south of Africa. Counterclockwise from upper left are shown the Brimstone canary, white-throated canary, yellow canary, and streaky-headed seedeater. The gray area is the distribution range.

FIG. 6.—Relict canaries include the Tibetan siskin (left), now existing in Eastern Himalayas to West China, and the streaky seedeater, now existing in patches of East Africa, Sudan, and Ethiopia to Northern Zimbabwe (right); note that the streaky seedeater is not classified with the big African canaries, although it is a large canary now living in Africa. Both species are supposed to have existed since about 9 MYA (fig. 2); however, their relationship may be loose, according to bootstrap values of node f, figure 1*a* and *b*.

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