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Research Article

Phylogeography of crossbills, bullfinches, grosbeaks, and rosefinches

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Abstract. Mitochondrial cytochrome b (cyt b) from 24 Carduelini species including crossbills, bullfinches, grosbeaks, rosefinches, and other related, but not conclusively classified species, was sequenced. These sequences were also compared with all the available sequences from the genera *Carduelis*, *Serinus*, and *Passer*. Phylogenetic analyses consistently gave the same groups of finches and the calculated divergence times suggest that speciation of the studied species occurred between 14 and 3 million years ago (Miocene-Pliocene), appearing before the *Passer*, *Carduelis*, and *Serinus* genera. Pleistocene glaciations may have been important in subspeciation. Crossbills are integrated within the genus *Carduelis*, together with redpolls; the common crossbill shows subspeciation with *Loxia japonica* in the Pleistocene epoch. *Pinicola enucleator* groups together with bullfinches and is probably the ancestor of the group. Hawfinch is only distantly related to the studied groups, and might either represent an isolated genus or be related to the New World genus *Hesperiphona.* The grosbeak genera *Eophona* and *Mycerobas* are clearly sister groups, and species belonging to the former might have given rise to *Mycerobas* species. The isolated (in classification) *Uragus sibericus* and *Haematospiza sipahi* are included within the genus *Carpodacus* (rosefinches); *Carpodacus nipalensis* is outside the genus *Carpodacus* in the molecular analyses and might be an isolated species or related to the genus *Montifringilla*.

Key words. Crossbill; bullfinch; grosbeaks, rosefinch; goldfinch; redpoll; Carduelini; canary; sparrow; greenfinch; siskin; linnet.

The Order Passeriformes includes the Passerine birds, and contains several families and subfamilies representing about one-half of extant birds [1]. The classification, phylogeny, and relationships of the so-called Carduelini finches remain unresolved. The tribe Carduelini includes goldfinches (genus *Carduelis*), crossbills (*Loxia)*, canaries *(Serinus*), rosefinches (*Carpodacus*), bullfinches (*Pyrrhula*) and grosbeaks (*Eophona, Mycerobas*) and some other related genera comprising one or two species [1]. DNA hybridization techniques have placed Carduelini finches closely to Hawaiian honeycreepers [1]. However, this technique presents many difficulties in interpretation and the Carduelini have also been related to ploceids and estrilids by osteological parameters [2] and to chaffinches, bramblings, and honeycreepers using myological data [3].

In the present work, we aimed to study the phylogenetic relationships among Carduelini (particularly crossbills, bullfinches, grosbeaks, and rosefinches) at the microevolutionary level by using mitochondrial cy-

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tochrome b (mt cyt b) DNA sequences. Comparison of these species together is important to obtain a detailed overview of these still not clearly classified groups of genera and species. The results are compared with the previously studied relationships among goldfinches [4] and canaries [5].

The present study examined the cyt b gene from the following genera: *Carduelis, Serinus, Loxia, Pyrrhula, Carpodacus, Mycerobas, Eophona, Haematospiza, Uragus,* and *Coccothraustes,* and used it as a source of systematic characters to test hypotheses of relationships among these genera. Particular attention was paid to terminal dendrogram branching in order to accumulate details necessary for a microevolutionary study.

Material and methods

Names of species and place of origin are given in table 1, which also includes GenBank accession numbers. Blood from living birds was drawn after their claws had been locally anesthetized with a lidocaine ointment and then cut. Blood was collected in ice-cold EDTA and frozen until use. 924 base pairs of the mt cyt b gene were amplified with primers L14841 5′-AAAAAGCTTCCAT CCAACATCTCAGCATGATGAAA-3′ and H15767 5′-ATGAAGGGATGTTCTACTGGTTG-3′ as detailed by Edwards et al. [6]. Polymerase chain reaction (PCR), cloning, and automatic DNA sequencing were performed as previously described [6, 7]. Two individuals per species were studied in order to identify variation among individuals. At least four clones from two different PCRs were sequenced from each species. Eigth cyt b clones from each specimen were sequenced and an average of five of these were used to obtain a consensus sequence.

Four different phylogenetic tree-constructing methodologies were used [8]: unweighted parsimony, neighbor joining (NJ) with a maximum-likelihood genetic distance matrix, linearized NJ trees with a Kimura biparametric genetic distance, and unweighted pair group with arithmetic mean (UPGMA) with Kimura biparametric genetic distance matrix. The statistical significance of a particular sequence cluster was also evaluated by the confidence probability (CP) (CP = 1 – type 1 error) [9]. This com-

Table 1. List of species, origin and mt cytochrome b sequence identification.

Species	Mt cyt b sequenc	Sample region
1 Siskin *(Carduelis spinus)*	L76391	Madrid, Spain
2 Pine siskin *(Carduelis pinus pinus)*	U79020	Jackson, Wyoming, USA
3 Linnet siskin *(Carduelis notata notata)*	U79019	Chiapas, Mexico
4 Lawrence's goldfinch *(Carduelis lawrencei)*	L76392	San Diego, California, USA
5 Citril finch *(Serinus citrinella citrinella)*	L77872	Madrid Sierra, Spain
6 Goldfinch *(Carduelis carduelis parva)*	L76387	Madrid, Spain
7 Goldfinch *(Carduelis carduelis caniceps)*	L76388	Kathmandu, Nepal
8 Common redpoll *(Carduelis flammea flammea)*	L76386	Brussels, Belgium
9 Artic redpoll *(Carduelis hornemanni hornemanni)*	U83201	Cage bird, Antwerp, Belgium
10 Two-barred crossbill *(Loxia leucoptera bifasciata)*	AF342878	Siberia, Russia
11 Common crossbill *(Loxia curvirostra curvirostra*	AF342876	Alcalá de Henares, Spain
12 Common crossbill *(Loxia curvirostra japonica)*	AF342877	Beijing, China
13 Common rosefinch *(Carpodacus erythrinus roseatus)*	AF342883	Islamabad, Pakistan
14 Scarlet finch *(Haematospiza sipahi)*	AF342875	Kathmandu, Nepal
15 Eastern great rosefinch *(Carpodacus rubicilloides lucifer)*	AF342868	Kathmandu, Nepal
16 Long-tailed rosefinch *(Uragus sibiricus lepidus)*	AF365877	Beijing, China
17 Beautiful rosefinch *(Carpodacus pulcherrimus pulcherrimus)*	AF365878	Kathmandu, Nepal
18 White-browed rosefinch *(Carpodacus thura thura)*	AF342869	Kathmandu, Nepal
19 Palla's rosefinch *(Carpodacus roseus)*	AF342867	Beijing, China
20 Three-banded rosefinch *(Carpodacus trifasciatus)*	AF342870	Szechwan, China
21 House rosefinch *(Carpodacus mexicanus frontalis)*	AF342865	Los Angeles, California, USA
22 Dark rosefinch *(Carpodacus nipalensis nipalensis)*	AF342866	Katmandu, Nepal
23 Brown bullfinch *(Pyrrhula nipalensis nipalensis)*	AF342884	Katmandu, Nepal
24 Pine grosbeak *(Pinicola enucleator pacatus)*	AF342882	Novorsibirsk, Russia
25 Beavan's bullfinch *(Pyrrhula erythaca wilderi)*	AF342862	Beijing, China
26 Common bullfinch *(Pyrrhula pyrrhula cineracea)*	AF342886	Novosibirsk, Russia
27 Common bullfinch *(Pyrrhula pyrrhula iberiae)*	AF342885	Santander, Spain
28 Common bullfinch *(Pyrrhula pyrrhula griseiventris)*	AF342881	Beijing, China
29 Japanese grosbeak *(Eophona personata magnirostris)*	AF342872	Beijing, China
30 Chinese grosbeak *(Eophona migratoria migratoria)*	AF342871	Beijing, China
31 White-winged grosbeak *(Mycerobas carnipes carnipes)*	AF342880	Kathmandu, Nepal
32 Collared grosbeak *(Mycerobas affinis)*	AF342879	Kathmandu, Nepal
33 Chaffinch *(Fringilla coelebs coelebs)*	L76609	Madrid, Spain

Lonchura nycthemera (pheasant) and *Gallus gallus* (chicken) sequencies were obtained from [12] and [11], respectively.

plements the bootstrap values. The BLAST program was used for sequence alignment (http://www.ncbi.nlm.nih.gov/BLAST).

The linearized NJ tree was also obtained for estimating divergence times using the divergence between the chicken and pheasant as a molecular calibration point [10]. Times of species divergence are only rough estimates. Thus, the time scale for the linearized NJ tree was obtained by comparing cyt b of chicken [11] and pheasant [12], two species that diverged about 20 million years ago (MYA) [13] according to combined fossil and molecular comparison calculations. The trees shown in this paper were rooted with *Fringilla coelebs.*

Maximum parsimony (MP), NJ with maximun-likelihood distances, linearized NJ with Kimura biparametric distancesn and UPGMA with biparametric distance matrices were obtained with the PAUP*4.0b2 program, kindly provided by Swofford [14] and with the MEGA package program in the case of the linearized tree [15]. The following calculations were carried out: number of substitutions (synonymous and nonsynonymous), number of variable and phylogenetically informative sites, and the base composition according to codon position. Bootstrap values were calculated to test the topology robustness of trees [16]. Low bootstrap values have been included in cases when the same topology was supported by at least two different tree construction methodologies [17]. To further assess node robustness, the statistical CP of a particular sequence cluster was calculated using the MEGA program [15]. 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 to compare pairwise percent sequence divergence to pairwise transversion and pairwise transition divergence at first, second, and third codon positions; both *Gallus gallus* and *Fringilla coelebs* chaffinch were used in the saturation plots (fig. 1). Two different estimates of percent divergence were used; these serve as approximations of time since divergence: Kimura's two-parameter [18] 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 [19, 20]).

Domestic chicken (*G. gallus* [11]) was used as a distant outgroup in both UPGMA and NJ linearized trees. Similar tree topologies were obtained with either *Gallus* or the more closely related *F. coelebs* (not shown).

A LINTRE test was also performed to test whether a molecular clock exists among all lineages used in our analyses [9]; linearized trees were obtained using this test, reestimating the branch lengths under the assumption of a constant rate of evolution (i.e., a molecular clock), and examining whether these showed significant differences from the trees illustrated in figure 3 (see below). The computer software used for these calculations can be obtained from the web sites: ftp://ftp.bio.indiana.edu/molbio/evolve/lintr/ or http://cib.nig.ac.jp/dda/ntakezak.html.

Results and discussion

Patterns of DNA base substitution

Saturation plots for cyt b (fig. 1) indicated that only third-position transitions showed a clear leveling-off associated with saturation; this occurred at 13% uncorrected total sequence divergence (crossbills, rosefinches, bullfinches, and grosbeaks/*Fringilla*) and at sequence divergences of more than 20% in the comparisons of crossbills, rosefinches, bullfinches and grosbeaks/*Gallus* (see fig. 1). Assessment of saturation gave similar results using Kimura's two parameter distances. We concluded that five out six data partitions (first, second, third codon position bases and transitions/transversions) were not saturated and were thus available to calculate correct phylogenies. Variable and phylogenetically informative sites were also calculated; there are 328 and 250 respectively, when crossbills, rosefinches, bullfinches, and grosbeaks are analyzed using *F. coelebs* as an outgroup, and 369 and

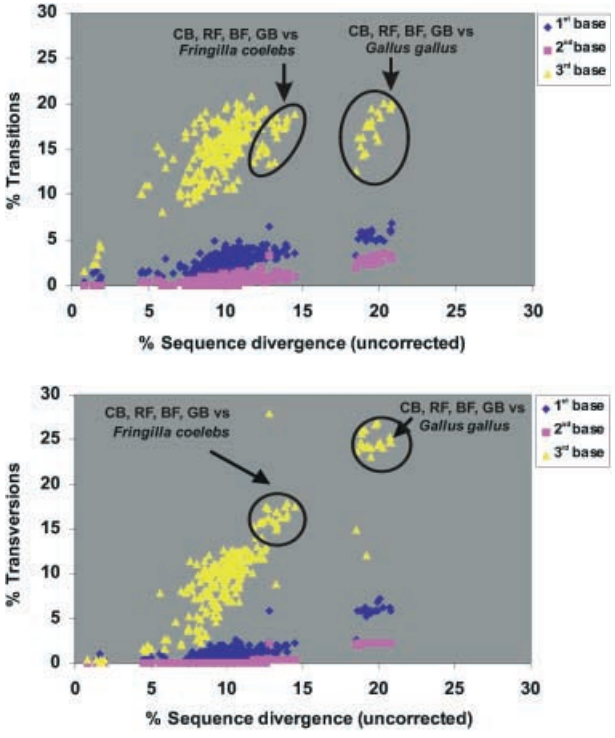

Figure 1. Saturation plots for the cytochrome b gene that relate uncorrected sequence divergence to changes due to transitions (top) and transversions (bottom) at first, second, and third codon positions. CB, crossbills; RF, rosefinches; BF, bullfinches; GB, grosbeaks. *Pinicola enucleator* is included among bullfinches and *Haematospiza sipahi* and *Uragus sibiricus* is included among rosefinches.

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250, respectively, when *G. gallus* is used as an outgroup. These numbers are appropriate to establish sound phylogenetic comparisons [21]. Data related to *Gallus* are included throughout the text when discussing the species time of divergence hypotheses.

The nucleotide distribution pattern of the cyt b gene of the birds under study was similar to that found in a previous analysis of this gene in other birds and mammals [13]. At the first codon positions, the four bases had similar frequencies; at the second position, fewer G residues and more T residues were seen. At the third codon position, the bias against G and T was strong, as demonstrated in previous studies [6, 13]. This bias in base composition was similar in all species studied (results not shown). Thus, the parsimony methodology seems to be adequate for all species studied [22]. The variability within the cyt b gene was theoretically sufficient to establish phylogenetic relationships according to the number of observed phylogenetically informative sites [21]; most of the differences were silent substitutions, as expected for a protein-coding gene, particularly for close relatives, such as species within a single genus [23].

As expected for a gene evolving relatively rapidly under strong functional constraints, 84.1% of the third codon positions were variable among species. The corresponding figures for the first and second positions were 18.5% and 3.9%, respectively. The base composition according to codon position was similar in all species; thus, a correct phylogeny may be inferred from the parsimony analysis.

The relative tempo of crossbill, rosefinch, bullfinch and grosbeak evolution was calculated. To estimate the rate of evolution, we used the type of calculations previously employed by ourselves and others [4, 5, 10]. After calibration of evolutionary rates, the times of origin of Darwin's finches, canaries, and goldfinches were estimated. An UPGMA dendrogram (not shown) and a linearized NJ dendrogram with Kimura biparametric distances (fig. 2) were constructed because these types of phylogenetic tree are more suitable for estimating divergence times than

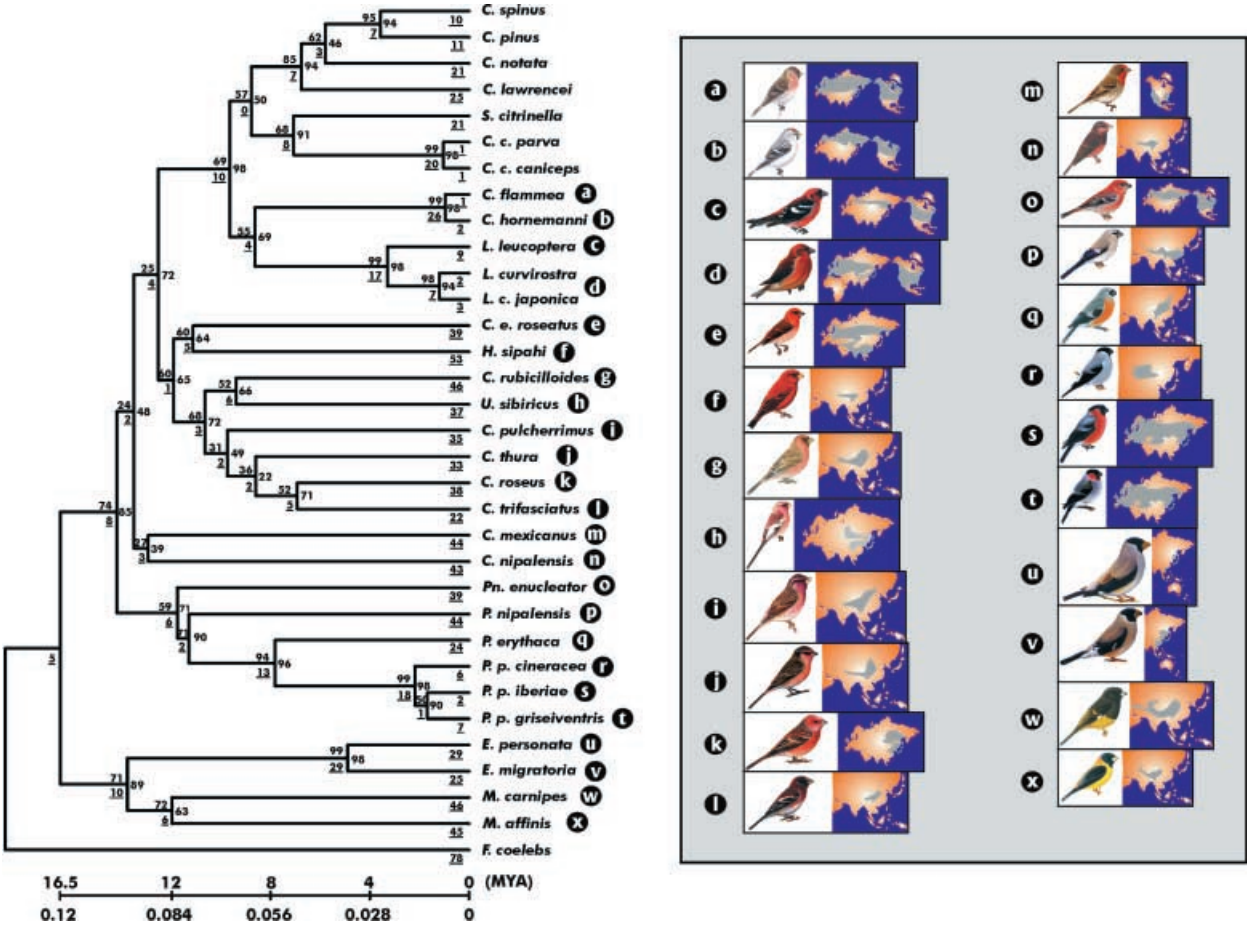

Figure 2. Linearized NJ tree calculated with Kimura biparametric distances. Divergence times are estimated for mt cyt b genes under a mixed molecular and fossil hypothesis [10]. The confidence probability (CP) is shown in the interior part of the node. Bootstrap values are shown above branches; branch lenghts (× 1000) are shown underlined below branches. Bird species used are detailed in table 1. Tree length (× 1000) = 1082. Genetic distances are depicted under the time scale. [Birds are modified from ref. 30. Maps were drawn in our laboratory using refs 30 and 34.

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other methods, in particular when a molecular clock exists (shown in this case by the LINTRE test, see below) [9]. Both the UPGMA and the linearized NJ trees were obtained using *F. coelebs* as an outgroup. Analysis of the genera *Loxia, Carpodacus, Uragus, Haematospiza, Pyrrhula, Eophona,* and *Mycerobas* suggested an evolutionary rate of 0.44×10^{-9} nonsynonymous substitutions per nonsynonymous site per year and 1.43×10^{-8} synonymous substitutions per synonymous site per year, so that the overall rate is $3.6 \pm 0.14 \times 10^{-9}$ [10]. This results in a substitution rate of 0.4% per million years, which leads to a rough approximation of 8% nucleotide substitutions per 10 million years. Taking into account the mtDNA differences found between the most distant species in the scaled constructed linearized tree shown in figure 2, which also approximates to 8%, the radiation seems to have started about 13 MYA, before that of the *Serinus* and *Carduelis* genera (fig. 2) [refs 4, 5 and unpublished data]. *F. coelebs* was found to have diverged 16.5 MYA; this value was used in figure 2. The topology of the UPGMA and linearized NJ trees (fig. 2) was very similar when using chicken and pheasant as additional outgroups (not shown). This is important for discussing the divergence times hypotheses put forward in the present work. However, the substitution rate found by us (0.4% per million years) differs from the standard 2% per million years. Furthermore, cranes show a slower rate of nucleotide substitution (about 1% [24]), closer to our results for *Carduelis, Serinus* and *Passer.*

Estimates using chicken/pheasant as an outgroup were similar to those obtained when *Fringilla* was the outgroup. Nevertheless, the calculation of times of species divergence needs to be confirmed by other methodologies and with additional species. Passerines [8] are found to be older than paleognathous birds, and the Carduelini species (*Carduelis* and *Serinus*) which are relatively close to Old World sparrows (genus *Passer*) are considered nearly as old as *Passer* [4, 5, 25, 26]. The use of either chaffinch or chicken and pheasant as outgroups for inferring phylogenies in our sample would seem to be correct, because only third position transitions appear to be saturated (fig. 1), but we chose the more closely related *Fringilla* to root the trees; the LINTRE computer program [9] also showed that there the evolutionary rates are constant among the bird lineages used. Thus, for cyt b, a molecular clock exists among the studied species (table 1). Finally, a caveat should be added because an early timing for passerine divergence [4, 5, 8, 27] is not accepted by others who propose speciation during the Pleistocene [28].

Phylogeography of crossbills, bullfinches, grosbeaks, and rosefinches

In the present work, the complete geographical range of crossbills, bullfinches, grosbeaks, and rosefinches was covered with the studied species.

In preliminary analyses (data not shown), canaries (genus *Serinus*) were shown not to be related to either goldfinches (genus *Carduelis*) or any of the genera newly studied in the present paper.

1) *Crossbills* (genus Loxia, 4 species)

Crossbills are closely related to redpolls (*Carduelis flammea* and *C. hornemanni*) in all analyses and all dendrograms (figs. 2, 3). Phylogenetic trees which included all *Carduelis* [4] and *Serinus* [5] species showed the same strong close redpoll/crossbill relatedness (data not shown). Like redpolls, crossbills have a northern hemisphere distribution, and a characteristic crossed mandible for specialized extraction of conifer seeds. They seem to have a more ancient origin than redpolls (see NJ linearized tree, fig. 2). They probably originated from a *Carduelis*-like ancestor when conifers were very common on Earth. Pine cones undergo irregular cycles of appearance and redpolls may have evolved at a time when pine cones were scarce and the hypothetical ancestor of the redpoll was forced to emerge from the conifer woods to find food in neighboring small mesothermal plants. The time when this occurred is uncertain; if we take the time scale hypothesized in figure 2, it would be about 9 MYA. There was clearly a decline in the number of conifers after the cold periods of Pleistocene glaciations, when redpolls could have appeared (fig. 2). However, the time scale is still debated [4, 5, 8, 27, 28]. Beak shape may change very rapidly according to feeding needs (i.e., lack of conifers [29]).

The Arctic redpoll (*C. hornemanni*, size 14 cm) is very similar to *Loxia curvirostra japonica*, a very small *Loxia* subspecies (14–15 cm). Red color varying in distribution placements and intensity according to subspecies, is conserved in both crossbill and redpoll males [30]. Typical seasonal north-south migrating patterns occur in both crossbills and redpolls, but the characteristic southern irruptive behavior of the former is unique, since the availability of pine cones is unpredictable in the pine woods [30, 31].

Consideration should also be given to the possibility of classifying redpolls apart from the genus *Carduelis* and together with *Loxia*, since redpolls are genetically distant from the twite/linnet couple [4]. Previously, twite, linnet, and redpolls were considered as a subgroup (or even another genus, *Acanthis*) [30, 31] within the genus *Carduelis*.

2) *Bullfinches* (genus *Pyrrhula*, 6 species)

These finches have a palearctic distribution, including the Azores Islands and Japan. In the present work, we collected species and subspecies representing the entire geographic range. Their beak has evolved to eat buds [30]. They cluster together with pine grosbeak, *Pinicola enucleator* (presently placed in the genus *Pinicola*, 2 species)

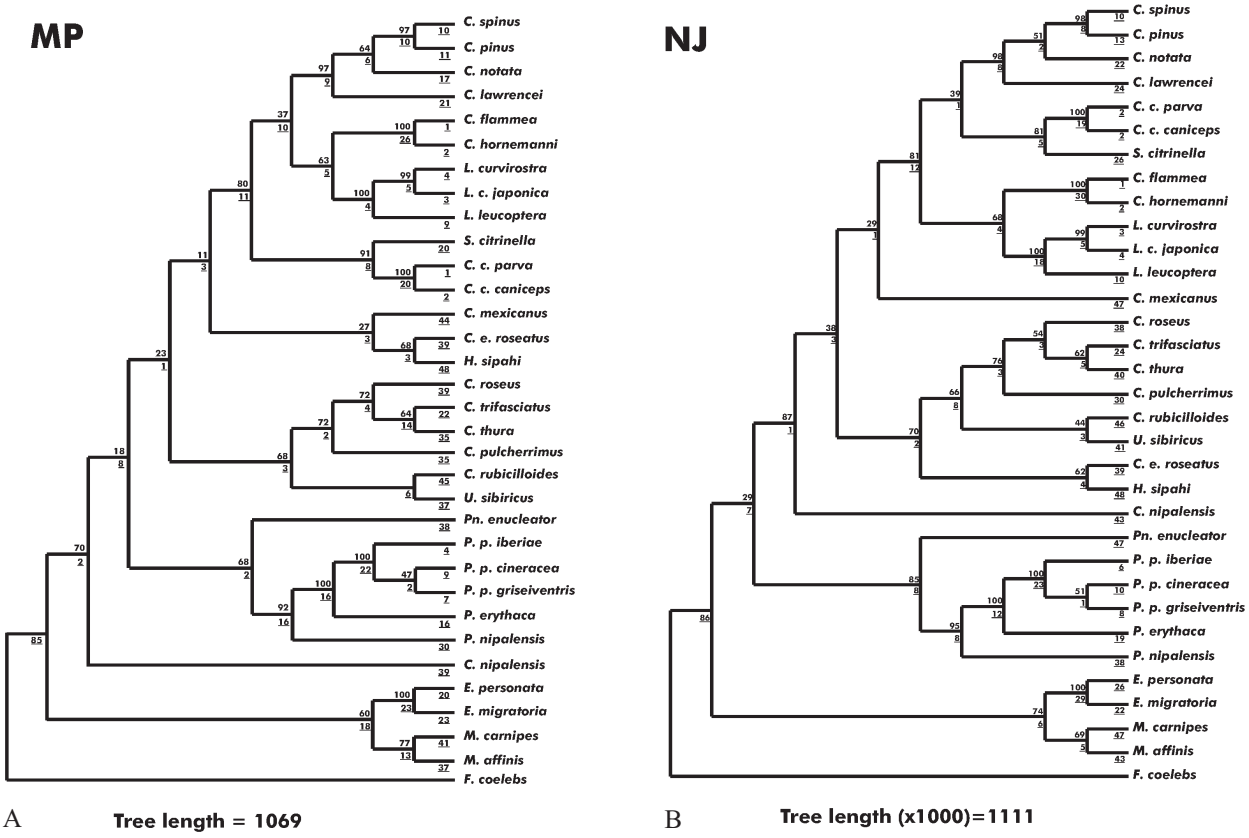

Figure 3. (*a*). Maximum unweighted parsimony tree. A Heuristic search was used (PAUP). Consistency and retention indexes were 0.42 and 0.53, respectively. The majority-rule bootstrap consensus tree based on 924 bases of mt cyt b genes is shown. The transition/transversion ratio was 2:1; the observed 5:1 ratio made no differences. Parsimony bootstrap analysis was done with 1000 replications, and values (in percent) are shown above branches. Parsimony was used unweighted (first, second, and third nucleotide) because weighting is only recommended for greater amounts of evolutionary divergence, which are not expected among the relatively closely related species analyzed [21]. The number of events is shown underlined below branches. Bird species used are detailed in table 1. (*b*) Neighbor-joining bootstrap tree (1000 replications) based on 924 bases of cyt b genes. Bootstrap values are shown above branches; branch lengths (× 1000) are shown underlined below branches. The evolutionary model used was 'the minimum evolution.' The transition /transversion ratio was 2:1. Distance matrices were calculated based on maximum-likelihood analysis [14]. Bird species used are detailed in table 1.

and monophyly of the group seems to be evident, since both the bootstrap and the CP of the node are significantly high (figs 2, 3). Therefore, *Pinicola* is not related to *Loxia* or *Carpodacus* as previously thought [30] and probably shares a common antecessor with bullfinches (fig. 2). Pine grosbeak would be the one *Pyrrhula* finch, found in North America, and showing a holoarctic distribution.

Pine grosbeak has been grouped with the red-headed rosefinch (*Pinicola subhimachala*) based on phenotypic characters in the genus *Pinicola* (only these 2 species). Both are larger (length ca 22 cm) than *Pyrrhula* and *Carpodacus* finches. However, the genus *Pinicola* might be split between *Pyrrhula* (pine grosbeak) and *Carpodacus* (red-headed rosefinch), the latter sharing many phenotypic characteristics with *Carpodacus* (rosefinches) except for the much bigger size (length 20 cm). Size should not be a criterion for including the red-headed rosefinch within the genus *Carpodacus,* since *Haematospiza sipahi* (19 cm in length) is a sister species of *Carpodacus erythrinus roseatus* (figs 2, 3). The phenotypically defined subspecies *Pyrrhula pyr. cinerea* and *griseiventris* are concordant with the molecular subspecies status suggested in this work (see fig. 2). The time of divergence of the *Pyrrhula* species (11 MYA) seems to be more recent than that of grosbeaks and rosefinches but earlier than the crossbill-redpoll divergence time.

3) *Grosbeaks* (genus *Coccothraustes*, 1 species; genus *Eophona*, 2 species; genus *Mycerobas*, 4 species)

Coccothraustes coccothraustes (hawfinch) was phylogenetically unrelated to any of the tested Carduelini groups [30]. To test hawfinch relatedness to the other groups newly studied in the present work, a 306-nucleotide-long cyt b sequence was retrieved from GenBank [26], aligned with the BLAST program, and NJ, UPGMA, and maximum-parsimony trees were constructed. Hawfinch remained as an isolated branch between the *Pyrrhula* and

Mycerobas genera and all other species represented in figure 2 and 3. It showed the same placement in all trees (data not shown). The genetic distances (maximum likelihood) from hawfinch to *Loxia*, *Pyrrhula*, *Carpodacus, Mycerobas*, and *Eophona* were relatively high and very similar (between 0.09–0.12). Mean distances between *Carduelis*, *Pyrrhula*, *Carpodacus, Mycerobas* and *Eophona* were placed between 0.001 and 0.03. Therefore, hawfinch molecular phylogeny supports the classification as a single and separate species from other Carduelini finches. However, the entire mt cyt b DNA gene should be tested for further assessment of hawfinch phylogeny, particularly with possible relatives, such as the New World *Hesperiphona* finches [30].

The genera *Eophona* and *Mycerobas* are closely related in all trees with high bootstrap and CP values (figs 2, 3). Whether they should be considered a single or two different genera is unclear, and the two missing *Mycerobas* species (*M. icteroides* and *M. melanozanthos*) should be tested in the future. The phenotypical relationship between *Mycerobas* and *Eophona* genera had been established previously [30].

Grosbeaks seem to have appeared on Earth earlier than the genera *Carduelis*, *Loxia*, *Carpodacus* and *Pyrrhula* in accordance with the hypothesized timing in figure 2 (14 MYA).

4) *Rosefinches* (genus Carpodacus, 21 species)

Most rosefinches are closer to the genus *Carduelis* than the others birds studied here. *Carpodacus nipalensis* (dark rosefinch) is an outlier from the group. The head, beak, and body characteristics are more like those of a chaffinch (*Montifringilla* sp.) than a rosefinch (unpublished results). It clusters in figure 2 (but not in fig. 3) with the house finch (*Carpodacus mexicanus)* because they are both outliers and long branches may attract each other in this type of analysis [32]. Furthermore, it is remarkable that the only American species studied in this work (house finch) is an outlier; it is separate from Asian rosefinches and it seems more closely related to genus *Carduelis*. When more rosefinch species are added to the dendrograms (figs 2, 3), the house finch may possibly become more integrated in the *Carpodacus* group. It shows no relationship with Old or New World sparrows [33]. This is more doubtful for *C. nipalensis*, which has such different phenotypic characters, and its relationships with geographically closer *Montifringilla* species should be studied in the future. *Uragus sibiricus,* which was classified as the only species belonging to the genus *Uragus* because it has a rosefinch body and a *Pyrrhula* beak, is definitely a sister species of *Carpodacus rubicilloides.* It may have changed its beak from that of *C. rubicilloides* (or its ancestor) as an adaptation to eat mainly buds, like *Pyrrhula*. This is another example of beak change in a relatively short time (figs 2, 3) [29]. The relationship of *U. sibiricus* with *Urocynchramus pylzowi* (pink-tailed rosefinch) should be studied in the future because of their phenotypic similarities and close geographical range (more limited for the latter, in central China).

Conclusions

1) The time of appearance of the species of crossbills, bullfinches, grosbeaks, and rosefinches studied is suggested in figure 2; the species may have arisen between the Miocene and Pliocene. However, although concordant times have been found for *Serinus* and *Carduelis* [4, 5], these early timings for passerine emergence are accepted by some [8, 10, 27] but not by all [28] authors.

2) Crossbills (genus *Loxia*) are integrated within the genus *Carduelis*, together with redpolls. The common crossbill shows subspeciation with *L. japonica* in the Pleistocene epoch.

3) Pine grosbeak (*P. enucleator*) groups with bullfinches and is probably one of the most ancient finches of the genus *Pyrrhula*, together with *Pyrrhula nipalensis* (figs 2, 3).

4) Hawfinch (*C. coccothraustes*) is not related to any of the bird groups studied in the present work (in particular not with *Eophona* or *Mycerobas*, which were considered by some investigators to be grosbeaks together with the hawfinch [30]). The phenotypic separation of the genus *Coccothraustes* is supported and its probably close relationship with the New World *Hesperiphona* species should be studied. *Mycerobas* and *Eophona* birds are closely related, the latter being more ancient and possible the common ancestor to both genera.

5) The long-tailed rosefinch (*U. sibiricus*) is a sister species of *Carpodacus* and is not related to *Pyrrhula* species. Its probable relationship with *U. pylzowi* should be studied in the future.

6) The large *Haematospiza sipahi* is included within the genus *Carpodacus*.

7) *C. nipalensis*, a *Fringilla*- or *Montifringilla*-like finch, is not included within genus *Carpodacus*.

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