J Ornithol (2006) 147: 448–456
DOI 10.1007/s10336-005-0036-2

ORIGINAL ARTICLE

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Rhodopechys obsoleta (desert finch): a pale ancestor of greenfinches (*Carduelis* spp.) according to molecular phylogeny

Received: 17 November 2004 / Revised: 21 July 2005 / Accepted: 1 August 2005 / Published online: 7 December 2005

Abstract The evolutionary history of three out of four birds traditionally classified into the genus *Rhodopechys* birds has been studied by comparing their mitochondrial cytochrome b DNA sequence with that of greenfinches and other genus *Carduelis* finches. The desert finch (*Rhodopechys obsoleta*) or a sister extinct species seems to have existed about 6 million years ago in Asian and perhaps African desert-like areas. This bird has no molecular relationship with other *Rhodopechys* birds and seems to have given rise to the greenfinches radiation, probably by allopatry of marginal or isolated groups; the latter would have evolved to green plumage colours and more simple song modulations (i.e., greenfinches). The possible role of assortative mating and the newly postulated acquired phenotypic characters in greenfinches speciation are discussed.

Keywords *Carduelis* · Desert finch · mtDNA · Phylogeny · *Rhodopechys* · Greenfinch

Introduction

Songbird evolutionary histories have been broadly studied (Grant and Grant 1997). Sometimes, molecular and phenotypic evolution are not concordant (Sibley and Ahlquist 1990). The ecological convergence of morphological and behavioural characters may lead to shared features among non-closely genetically related species occurring in similar or quasi-identical environments and, conversely, disparate features may occur among genetically sister taxa thriving under different conditions along generations. Therefore, some traits may not be useful for tracing the evolutionary histories under study.

Mt cyt b (mitochondrial cytochrome b) gene DNA sequencing has been widely used in molecular systematics. This gene has proved to be helpful in defining evolutionary relationships among relatively distant and closely related birds, even at the subspecies level (Questiau et al. 1998; Friesen et al. 1996). Songbird genera (about 6,000 worldwide species) have been thus surveyed; also superfamilies and other groups have been more precisely defined, such as (1) *Corvoidea* [logrunners (Norman et al. 2002), ravens (Omland et al. 2000), crows (Kriukov and Suzuki 2000), vireos (Cicero and Johnson 1998), African monarchs (Pasquet et al. 2002)], (2) *Polioptilidae* [gnatcatchers (Zink and Blackwell 1998)], *Sylvioidea* [reed warblers (Helbig and Seibold 1999), swallows (Whittingham et al. 2002), babblers (Cibois et al. 1999), crests and kinglets (Packert et al. 2003), tits (Salzburger et al. 2002a, b)], (3) *Menuroidea*, lyrebirds (Ericson et al. 2002), and (4) *Passeroidea* [siskins (Arnaiz-Villena et al. 1998), canaries (Arnaiz-Villena et al. 1999), and others members of tribe *Carduelini* / *Emphasis>* (*Arnaiz-Villena et al. 2001*), *Old-World sparrows* (*Allende et al. 2001*), *tanagers* (*Burns 1997; Hackett 1996*), *towhees* (*Zink et al. 1998*), *pipits* (*Voelker 1999*), *longspurs and snow buntings* (*Klicka et al. 2003*), *warbling-finches* (*Lougheed et al. 2000*), *Darwin's finches* (*Sato et al. 1999, 2001*) *as well as many others groups of the Fringillidae* family (Yuri and Mindell 2002)].

In the present paper, we study the evolutionary history of greenfinches (*Carduelis*), a group of Eurasian species (Table 1), by using mt cyt b DNA sequences, and their possible origins related to a desert or a semi-desert land based finch (*Rhodopechys obsoleta* or desert finch), traditionally included within genus *Rhodopechys*

Communicated by A. Helbig

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Table 1 List of species. Origin and sequence identification (*GeneBank accession number*)

Carduelis and *Rhodopechys* spp.	Mt cyt b sequence	Sample region
Siskin (*Carduelis spinus*)	L76391	Madrid, Spain
Pine siskin (*C. pinus pinus*)	U79020	Jackson, WY, USA
Red siskin (*C. cucullata*)	L76299	Venezuela[a]
Yellow–bellied siskin (*C. xanthogastra xanthogastra*)	L76389	San José, Costa Rica
Olivaceous siskin (*C. olivacea*)	L77871	Lima, Perú
Black siskin (*C. atrata*)	L76385	Sucre, Bolivia
Thick-billed siskin (*C. crassirostris crassirostris*)	L77869	Mendoza, Argentina
Hooded siskin (*C. magellanica magellanica*)	U79016	Misiones, Argentina
Andean siskin (*C. spinescens spinescens*)	U79017	Mérida, Venezuela
Yellow-faced siskin (*C. yarellii*)	U83200	Recife, Brasil[b]
Black-chinned siskin (*C. barbata*)	L77868	Magallanes, Chile
Black-headed siskin (*C. notata notata*)	U79019	Chiapas, México
Linnet (*C. cannabina cannabina*)	L76298	Madrid, Spain
Twite (*C. flavirostris flavirostris*)	U83199	Cage bird, Antwerp, Belgium[c]
Dark-backed goldfinch (*C. psaltria hesperophila*)	L76390	Sacramento, Calif., USA
Dark-backed goldfinch (*C. psaltria columbiana*)	U78324	Maracay, Venezuela[d]
American goldfinch (*C. tristis salicamans*)	U79022	San Francisco, Calif., USA
Llawrence's goldfinch (*C. lawrencei*)	L76392	San Diego, Calif., USA[e]
Common redpoll (*C. flammea flammea*)	L76386	Brussels, Belgium
Arctic redpoll (*C. hornemanni hornemanni*)	U83201	Cage bird, Antwerp, Belgium
Goldfinch (*C. carduelis parva*)	L76387	Madrid, Spain
Goldfinch (*C. carduelis caniceps*)	L76388	Katmandú, Nepal
Greenfinch (*C. chloris aurantiventris*)	L76297	Madrid, Spain
Oriental greenfinch (*C. sinica sinica*)	L76592	Szechwan, China
Black-headed greenfinch (*C. ambigua ambigua*)	U78322	Szechwan, China[f]
Himalayan greenfinch (*C. spinoides spinoides*)	U79018	Katmandu, Nepal
Desert finch (*R. obsoleta*)	AF342889	Kabul, Afghanistan
Trumpeter finch (*R. githaginea*)	AF342887	Gran Canaria Island, Spain
Mongolian trumpeter finch (*R. mongolica*)	AF342888	Gilgit, Pakistan
Chaffinch (*F. coelebs coelebs*)	L76609	Madrid, Spain

[a]Ascents from Venezuela; this particular specimen was bred in Madrid as a cage bird
[b]Ascents from Recife (Brasil), but this particular specimen was bred in Reggio nellÍEmilia (Italy)
[c]Ascents originating in Northern Europe emigrated to the Antwerp region in winter
[d]Phenotypes of *C. psaltria* from Colorado and from Venezuela are not easily distinguishable. See also (Clement et al. 1993). All specimens studied are male, except for those indicated by [e]
[e] undetermined sex
[f]Note that isolated *C. monguilloti* (Vietnamese greernfinch), phenetically close to *C.ambigua* (Clement et al. 1993) has not been studied
Chicken and pheasant sequences were obtained from Desjardins and Morais (1990) and Kornegay et al. (1993) ,respectively. *Rhodopechys* and greefinches DNAs are frozen in our files and are available under the numbers: 0217 (*R. obsoleta*), 0010 (*R. githaginea*), 0765 (*R. mongolica*), 0006 (*C. Chloris*), 0064 (*C. sinica*), 0166 (*C. spinoides*), 0117 (*C. ambigua*)

by phenotypic methodologies (Fig. 1a, b). *Rhodopechys* species (Table 1) thrive at present in African and Central Asian deserts or very arid areas and show pale or sandy coloured wings and bills (Fig. 2); they are thought to have a Central Asian origin (Clement et al. 1993). In contrast, greenfinches (Fig. 2) show colourful bright yellow and olive green colours, and inhabit Western Europe (*Carduelis chloris*) and Eastern Asia (*C. sinica*, *C. ambigua* and *C. spinoides*). They occur in a variety of areas ranging from deciduous or conifer forests to scrub, cultivated and urban areas. In the present paper, *Rhodopechys* spp. (all but *R. sanguinea*) have been sequenced for their mt cyt b DNA and compared with the orthologous greenfinch gene. We conclude that *R. obsoleta* bordering populations might have dispersed during Pliocene and Miocene Epochs giving rise to *C. chloris*, *C. sinica*, *C. ambigua* and *C. spinoides* in Eurasian habitats (forests or non-dry plains). These molecular based results will be discussed on the bases of the presently found phenotypic characters in these species.

Methods

Bird samples

Names of species and place of origin are given in Table 1. Blood from living birds was drawn after their claws were locally anaesthetized with a lidocaine ointment and then cut; birds were also photographed. Blood was collected in ice-cold EDTA and frozen until use. 924 base pairs (from 97 to 1,020) of the mt cyt b gene were amplified with primers L14841 5′-AAAAAGCTTCCATCCA ACATCTCAGCATGATGAAA-3′ and H15767 5′-ATGAAGGGATGTTCTACTGGTTG-3′ as detailed by Edwards et al. (1991). Polymerase chain reaction (PCR), cloning and automatic DNA sequencing were performed as previously described by Arnaiz-Villena et al. (1992) and Edwards et al. (1991). At least, four clones from each of two different PCRs were sequenced from each species. All clones studied in the present paper gave the same sequence.

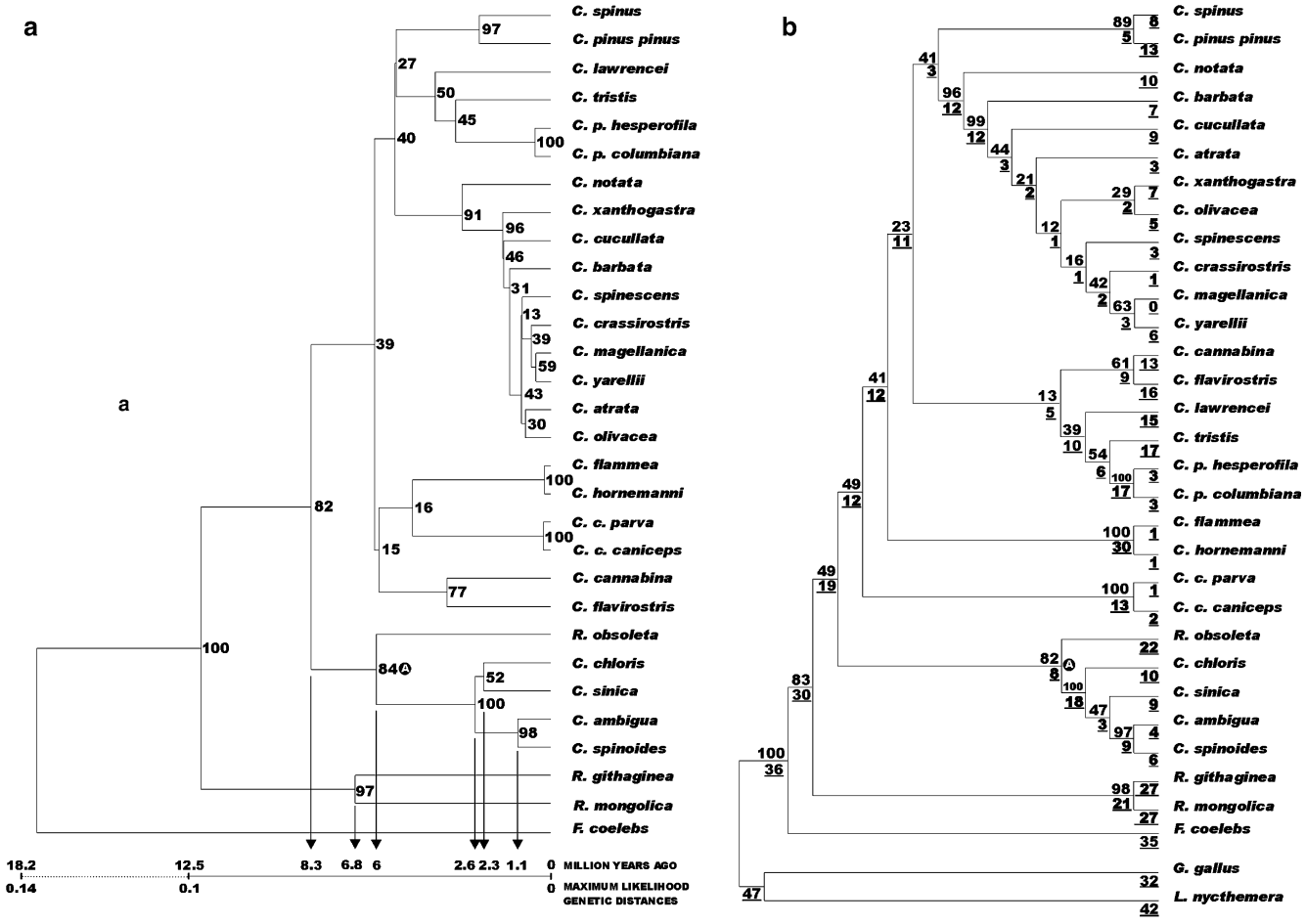

Fig. 1 **a** Maximum likelihood-based tree showing approximate calculations on the time of appearance of genera *Carduelis* and *Rhodopechys* lineages. This linearized tree was constructed by assuming that evolutionary rates between lineages may be different (Thorne et al. 1998). PARAMCLOCK PAUP command was used for tree building. Divergence times were estimated assuming an evolutionary rate of 0.8% substitutions per site and per million years, found by Fleischer et al. (1998). This rate is based on the cyt b sequence divergence of Hawaiian drepanidines, and external geological calibration points. Groups of taxa are similar to those obtained in the parsimony (see **b**) and NJ with ML genetic distance dendrograms (tree not shown). Genus *Carduelis* speciation seems to have occurred during the Miocene and Pliocene epochs in both the Northern and Southern Hemispheres (Arnaiz-Villena et al. 1998). *R. mongolica* and *R. githaginea* cluster separately from *R. obsoleta*, which appears as the greenfinch ancestor. One thousand replication bootstrap values are depicted in the interior part of the nodes. ML-based tree scores: tree length (1,000 times) = 1,055; ln Likelihood = −4,392.98019 estimated transition/transversion ratio = 4.61. ML (1,000 times) genetic distances are depicted above the time scale (million years ago). ML analysis settings were: two substitutions types; estimated transition/transversion ratio via ML; HKY85 model; empirical nucleotide frequencies; none assumed proportion of invariable sites and gamma distribution of rates at variable sites, divided in four categories as done by Yang (1994) for mitochondrial DNA sequences. **b** Parsimony tree. Branch length and 1,000 replication bootstrap values (Felsenstein 1985) are underlined below and above the branches, respectively. The addition of sequences was determined by the closest stepwise addition. TBR (Tree Bisection and Reconnection) branch swapping was set in order to increase the probability of finding the optimum trees. The scores for the parsimony tree are: tree length = 720; consistency index = 0.497; retention index = 0.664. Chicken and pheasant sequences (distant outgroups) were take from Desjardins and Morais (1990) and Kornegay et al. (1993), respectively

Statistic analyses and dendrogram construction methods

The following calculations were carried out: base composition (also according to codon position), number of synonymous (*dS*) and non-synonymous (*dN*) distances by using the modified Nei-Gojobori method (Nei and Gojobori 1986) considering the estimated transition/transversion ratio via maximum likelihood (ML; Felsenstein 1981) and Jukes-Cantor distances (Jukes and Cantor 1969) to allow for multiple hits at the same site. Saturation plots (not shown) were carried out in order to be aware of transitional changes that may become saturated (multiple substitutions at single site) and then uninformative at certain divergence times. Uncorrected pairwise divergence was used as an estimate of percent divergence [$P = n_d/n$, where P is the proportion sequence divergence between two sequences, n_d is the number of nucleotides that differ between two sequences, and n is the total number of nucleotides compared (Nei 1987)]; this gives an approximation of time of species divergence.

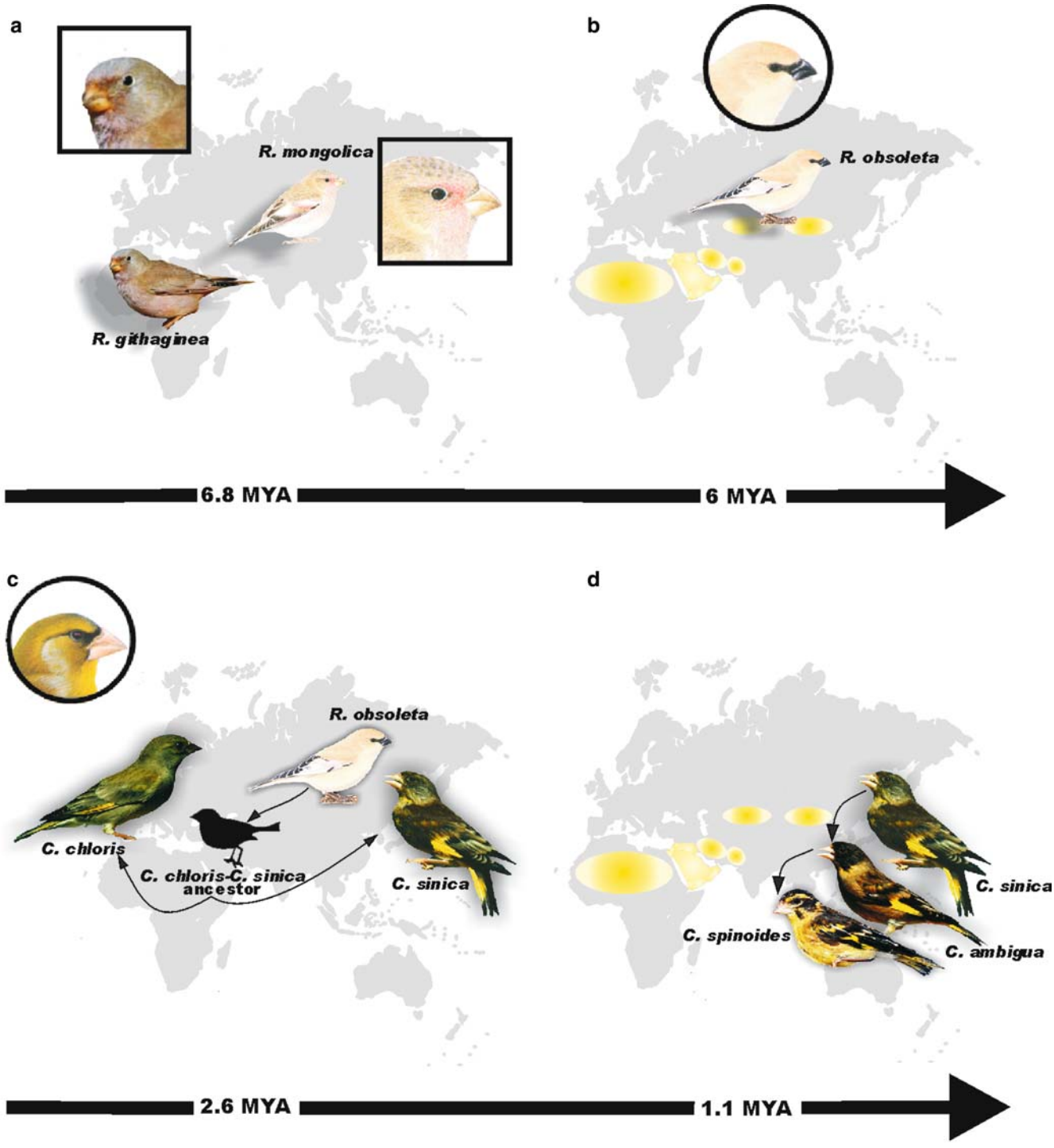

Fig. 2 Hypothetic model of greenfinch group evolution from semi-desert land based populations of *R. obsoleta*. Birds are placed approximately over their present distribution areas. Approximated times in million years for each dispersion are in accordance to Fig. 1a: **a** Molecular unrelatedness of *R. githaginea* and *R. mongolica*; **b** Apparition of *R. obsoleta* in desert areas (*yellow*); **c** Postulated dispersion of *R. obsoleta* populations that would have given rise to a *C. chloris*-*C. sinica* ancestor which would have evolved separately to reach different species status; **d** Postulated southward dispersions during Pleistocene glaciations: first *C. sinica* populations would disperse and give rise to *C. ambigua*, and, later, *C. ambigua* would have dispersed southward evolving to *C. spinoides*. *C. chloris* and *R. obsoleta*; the males' very dark eye-stripes are showed in the *circled insets* (more evident in breeding season). *Squared insets* show male birds without eye-stripes (*R.mongolica* and *R.githaginea*). Photographs belong to Dr. Arnaiz-Villena except for *R. githaginea* (Blake 2001). *Drawings* were taken from Clement et al. (1993)

Table 2 Enforced constraints on neighbour-joining (*NJ*) upon maximum likelihood (*ML*) distances and parsimony topologies support *R. obsoleta* inclusion within genus *Carduelis*

	Parsimony scores			NJ and ML scores	
	Tree length	Consistency index	Retention index	ln Likelihood	Minimum evolution score
Unconstrained phylogenetic tree (*R. obsoleta* within greenfinches, Fig. 1a)	466	0.50	0.66	−5,273.83	1.04
R. obsoleta within *R. githaginea* and *R. mongolica* cluster (tree not shown)	720[a]	0.47	0.60	−5,397.79	1.10

[a]Statistically significant differences according to the winning sites, Templeton, and Kishino and Hasegawa tests

Three phylogenetic inference criteria were used as implemented in PAUP software package (Swofford 2002): (1) parsimony (Fitch 1971), (2) distance-based methods, namely, Neighbour-Joining (NJ, Saitou and Nei 1987), and (3) ML-based tree (Felsenstein 1981); the PARAMCLOCK PAUP command was used. The characters were set unordered in the parsimony analysis. *Fringilla coelebs* was chosen as outgroup to root the trees following evidence from other authors (Groth 1998) and ourselves (Allende et al. 2001; Arnaiz-Villena et al. 1998, 1999, 2001; van den Elzen et al. 2001). Also *Gallus gallus* (chicken) and *Lophura nycthemera* (silver pheasant) were also used as more distant outgroups. Evolutionary rate calculations were carried out with MEGA v2.1 program (Kumar et al. 2001).

To estimate divergence times we assumed an evolutionary rate of 0.8% substitutions per site and per million years. This rate was found by Fleischer et al. (1998) in the Hawaiian drepanidines (subfamily Fringillini, tribe Drepanidini) based on an external geological calibration. Bearing in mind that variation of evolutionary rate among lineages may exist, we estimated the branch lengths by ML allowing rates to continuously change over time, according to the molecular clock model of Thorne et al. (1998). This model was successfully applied to several biological issues (Hasegawa et al. 2003, and references therein). See Fig. 1a for ML-based tree and footnote for ML analysis settings. PARAMCLOCK PAUP command was used to build the ML-based linearized tree.

The search of the most parsimonious trees (Fig. 1b) was heuristic because the number of taxa used (32) rendered an exhaustive one impractical. Parsimony settings are depicted in Fig. 1b footnote.

To discover more about the phylogenetic position of *Rhodopechys* spp. in relation to *Carduelis* spp., we constrained parsimony and NJ trees on ML distances (trees not shown). These tree topologies separated *Carduelis* and the three *Rhodopechys* spp. in two different monophyletic clusters; these were compared to another non-enforced tree (Table 2). The different indicators used to assess *Rhodopechys* spp. monophyly in the parsimony enforced trees were consistency and retention indexes, the winning sites test (Prager and Wilson 1988), the Templeton test (1983) and the Kishino and Hasegawa test (1989) that evaluate the tree lengths of the constrained parsimony topologies (genus *Rhodopechys* monophyly) compared to those of the unconstrained trees. In the NJ trees with ML distances we used tree length and likelihood values. The tests mentioned above were conducted with PAUP software package (Swofford 2002).

Results

Patterns of DNA base substitution

Saturation plots for cyt b mtDNA (not shown) indicated that only third position transitions showed a clear levelling-off associated with saturation; this occurred between pair species diverging from 9% (*Rhodopechys mongolica* and *R. githaginea* versus *Carduelis* spp.) to 12% (*F. coelebs*, *G. gallus* and *L. nycthemera* versus *Carduelis* and *Rhodopechys* spp.) of uncorrected total sequence divergence (Nei 1987). Therefore, it was concluded that five out of six data partitions (at the first, second, third codon position bases and transitions/transversions) were not saturated and were thus available to calculate reliable phylogenies (Hillis et al. 1994). Variable and phylogenetically informative sites were also calculated; these were 349 and 249, respectively, when the present studied *Carduelis* and *Rhodopechys* spp. group was analyzed using *F. coelebs* as an outgroup (Arnaiz-Villena et al. 1998). This variability within the cyt b gene was theoretically sufficient to establish phylogenetic relationships according to the number of observed parsimony-informative sites (Hillis et al. 1994).

The cyt b gene nucleotide distribution pattern, that is, the A, C, G and T percentages at the first, second and third codon position of the birds under study, was similar to that found in previous analyses of this gene for other birds (Hackett 1996; Krajewski and King 1996; Arnaiz-Villena et al. 1999) and mammals (Irwin et al. 1991): (1) the four bases had similar frequencies at the first codon positions, (2) fewer G residues and more T residues were seen at the second position, and (3) the bias against G and T was strong at the third codon position (Edwards et al. 1991). Thus, a reliable phylogeny may be inferred from the parsimony analysis. The overall bias in base composition was similar in all species studied (24.4%T, 34.3%C, 27.4%A, 13.9%G). There-

fore, the parsimony (Fitch 1971) and NJ (Saitou and Nei 1987) methodologies seemed to be adequate for studying all our species being tested (Lockhart et al. 1994). However, differences among overall A, C, G and T base frequencies within all studied species were found to be significant (chi-squared, $P < 0.05$). These differences led to using the HKY85 model (Hasegawa et al. 1985) (that assume the presence of unequal nucleotide frequencies) for the ML analysis and subsequent NJ tree construction. Nearly all sequence differences were silent substitutions, as expected (Kocher et al. 1989). Thus, 50.9% of the third codon positions were not conserved among species, as has been shown for this mt cyt b gene (evolving relatively rapidly under strong functional constraints); The variability for the first and second codon positions was 5.8 and 0.6%, respectively.

The analysis of the number of substitutions per site in the presently studied species was found to be: 0.1970 ± 0.0134 per synonymous, 0.0032 ± 0.0008 per non-synonymous and 0.0631 ± 0.0046 per total sites [the number after $\pm$ is the standard deviation computed by bootstrap methodology, as stated in the methods section (Felsenstein 1985)]. We also computed these figures by others' methods based on Kimura's 2-parameters (Li et al. 1985; Pamilo and Bianchi 1993; Kumar et al. 2001) obtaining similar results (not shown).

Genetic distances and phylogeny

Both, uncorrected *P* genetic distances as well as patristic distances (sum of steps on path between each pair of taxa) depicted in Table 3 show that: (1) greenfinches are a homogeneous group, and (2) *R. obsoleta* stands out as a species closer to them than to their believed counterparts *R. mongolica* and *R. githaginea*.

Parsimony, distance-based and ML-based trees

In general, with slight differences due to the different tree-constructing methodology used ML-based tree (Fig. 1a), NJ on ML distances (not shown) and parsimony trees (Fig. 1b) rendered the same branching pattern previously described by Arnaiz-Villena et al. (1998, 2001), mirroring the geographical distribution of genus *Carduelis* spp.

Enforced trees (constraint analyses)

The enforced monophyly of the three *Rhodopechys* spp. considered in the present work (Table 2) rendered a higher tree length and lower consistency and retention indexes in the parsimony analysis when compared with the unconstrained tree (Fig. 1b). The winning sites test (Prager et al. 1988), Templeton test (1983), and Kishino and Hasegawa test (1989) revealed a statistically significant differences between the lengths of the constrained and unconstrained parsimony trees (Table 2). Thus, the theoretical topology that would group *R. obsoleta* with *R. mongolica* and *R. githaginea* is supported by the data matrix to a lower degree than that obtained for greenfinches–*R. obsoleta* (Fig. 1). Regarding the NJ trees with ML distances (trees not shown), the enforced tree showed a negative branch length leading to the cluster grouping *Rhodopechys* spp., and therefore increasing the minimum evolution score; the likelihood value was also lower in the constrained tree (tree not shown) than that obtained for greenfinches *R. obsoleta*.

Timing of lineage splits

Regarding the *Rhodopechys* species, monophyly is not supported, either in parsimony or in distance-based tree (Fig. 1a, b, respectively); instead, they seem to belong to two different radiations. So, *R. githaginea* and *R. mongolica* would have arisen about 7 mya and, later, about 6 mya, within genus *Carduelis* radiation, it would be *R. obsoleta*. Indeed, its position in the trees is supported by bootstrap values as the greenfinch lineage ancestor (node A in Fig. 1a, b).

Discussion

Biogeographic pattern hypothesis

While *R. githaginea* and *R. mongolica* would have arisen about 6.8 mya (Fig. 2a), *R. obsoleta* (desert finch) seems to be a closely related ancestor of the greenfinches lineage arisen 6 mya (Fig. 2b), in the late Miocene epoch (node A in Fig. 1a). Indeed, about 7–5 mya, a glacial mantle ceased to cover Antarctica and Greenland and many Asian and African arid areas appeared (Uriarte-Cantolla

Table 3 Uncorrected *P* (*left*, in bold typing) and patristic (*right*) genetic distances matrix

	R. obsoleta		*C. chloris*		*C. sinica*		*C. ambigua*		*C. spinoides*		*R. githaginea*		*R. mongolica*	
R. obsoleta	–	–												
C. chloris	**5.8**	23	–	–										
C. sinica	**5.5**	22	**2.7**	11	–	–								
C. ambigua	**5.5**	25	**2.7**	12	**2.7**	13	–	–						
C. spinoides	**6.2**	27	**3.2**	12	**2.0**	11	**1.4**	6	–	–				
R. githaginea	**10.5**	46	**9.3**	47	**9.4**	50	**9.6**	53	**9.7**	49	–	–		
R. mongolica	**9.1**	37	**9.7**	54	**9.4**	53	**9.0**	50	**9.7**	54	**7.5**	19	–	–

Uncorrected *P* distances are given in percentages

2003). About 2.6 mya, when the weather became much warmer (Uriarte-Cantolla 2003) and during periods of severe drought, bordering populations of desert finch might have set out from semi-desert areas to more humid habitats and evolved to a greenfinch-like bird (extant or extinct ancestor). This ancestor may have differently evolved in Western Europe and Eastern Asia giving rise to *C. chloris* and *C. sinica*, respectively (Fig. 2c). Later, during the climate changes of the last 2 mya glaciations (Uriarte-Cantolla 2003), *C. sinica* populations may have dispersed southwards giving rise to *C. ambigua* and, finally, populations of this latter species would have also dispersed evolving to *C. spinoides* (Fig. 2d; and see also Arnaiz-Villena et al. 1998).

Whether *R. obsoleta* also existed in African deserts remains as a possibility. Also, an African *R. obsoleta* ancestor for the Mediterranean-European greenfinch (*C. chloris*) is not discarded.

Evolutionary pattern hypothesis

Taking into account the above mentioned approximate radiation patterns and timings (Fig. 1a, b), as well as the theoretical geographic and ecological scenarios (Fig. 2), the question arises as to how the speciation leading to greenfinches from a semi-desert-based species like *R. obsoleta* or a close extinct species would have proceeded. The answer could be related to the different environments in which greenfinches and desert finch might have occurred. Open habitats (i.e., deserts or arid-areas) inhabited by *Rhodopechys* spp., are thought to favour lower hue and lower bright values in plumage colorations than do other habitats (McNaught and Owens 2002). Sandy and pale coloured wings could be mirroring a convergent evolution to a desert environment adaptation between *R. mongolica*/*R. githaginea* and *R. obsoleta*. Conversely, *R. obsoleta* molecular sister taxa (greenfinches) bear olive green, bright green and bright yellow plumages that usually result from melanin and carotenoid pigment combinations (Gill 1999). These colorations may provide a better species-specific signalling performance in denser habitats such as forests or other non-arid areas. Melanins have been considered to protect feathers from bacterial degradation in humid habitats (Burtt and Ichida 2004), Thus, it is feasible that a higher melanin content may have been selected in populations settling down in forests or similar more humid habitats (i.e., in greenfinches). Other melanin related characters, like the eye-stripe present in both *R. obsoleta* and *C. Chloris* males, may contribute to sexual dimorphism and female mating choice in the breeding season (see head details in Fig. 2). This would suggest that the extant closest relative to *R. obsoleta* is *C. chloris*, which is the only one with a preserved black eye-stripe in the breeding season (Fig. 2). Carotenoids, the other major pigments responsible for plumage colorations (Gill 1999), are scarce in dry areas, and could have reduced to a minimum in the diet of species occurring in Central Asia or Africa areas undergoing desertification in Pliocene and Miocene Epochs. In this way, adaptive convergence to a sandy and pale colour plumage common in the traditionally recognised *Rhodepechys* species could have occurred in *R. obsoleta* via carotenoid shortage, among other environmental factors (Burtt and Ichida 2004).

On the other hand, the assortative mating selection may not only be restricted to differences in feather pigment content; species specific song may also influence the assortative mating (Grant and Grant 1997; Haavie et al. 2004) between allopatric populations (of *R. obsoleta*) which may later have differentiated into species (*R. obsoleta* and *C. chloris*). *R. obsoleta* song, although more harsh and nasal, is similar to those of *C. chloris* and the linnet (*Carduelis cannabina*, Europe and western Asia) (Erard and Etchécopar 1970). Thus, it is feasible that song pattern of *R. obsoleta* or *R. obsoleta*-like males settling away from deserts changed by taking over song variants of other species living close by in a forest-like environment (i.e., linnet). This may have helped *C. chloris* achieve full speciation.

Zusammenfassung

Der Weißflügelgimpel (*Rhodopechys obsoleta*): ein heller Vorfahr des Grünfinks entsprechend molekularer Phylogenie

Die evolutionäre Geschichte von drei der vier Arten, die traditionell der Gattung *Rhodophechys* zugerechnet werden, wurde durch Vergleich der DNA-Sequenz ihres mitochondrialen Cytochrom B mit der des Grünfinken und anderer Arten der Gattung *Carduelis* untersucht. Der Weißflügelgimpel (*R. obsoleta*) oder eine ausgestorbene Schwesterart scheint vor etwa 6 Millionen Jahren in asiatischen und vielleicht afrikanischen, wüstenartigen Gegenden gelebt zu haben. Diese Art hat keinen molekularen Bezug mit anderen Arten der Gattung *Rhodopechys* und scheint der Startpunkt der Grünfink-Radiation gewesen zu sein, vermutlich durch Allopatrie von marginalen oder isolierten Gruppen. Letzterer wäre dann hin zu einer grünen Gefiederfärbung und einfacherer Gesangsstruktur (d. h. Grünfinken) evoluiert. Die mögliche Rolle assortativer Verpaarung und die neu postulierten phänotypischen Eigenschaften in der Artbildung des Grünfinks werden diskutiert.

Acknowledgements This work was supported by grants from the Spanish Ministry of Science PM-1999-23 and BMC-2001-1299 Mufua Madrileña Automovilista, and was in agreement with all the laws valid in the countries where samples were taken. We are also indebted to the following Spanish ornithologists: Bernardino Yebes, Guillermo Cabrera Amat, Gloria Gardó, Francisco Mira Chinchilla, Arturo Fernandez Cagiao and Álvaro Guillén.

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