The phylogeographic differentiation of the European robin *Erithacus rubecula* on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin taxon on Gran Canaria?

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Sequences of the mitochondrial cytochrome b-gene (1125 basepairs) of the European robin, *Erithacus rubecula*, from the Canary Islands, Spain, revealed new insights into the systematics and the phylogeography of this taxon. Additionally, a range of morphological measurements was investigated by using discriminant function analysis. Genetic and morphological data show no differences between robins from the western Canary Islands and mainland Europe and these populations should be retained within the nominate form *E. r. rubecula*. Sequence data revealed well defined haplotypes and distinct genetic distances between *E. r. superbus*, both from Gran Canaria and Tenerife. *E. r. superbus* from Gran Canaria takes a more basal position and birds from Tenerife are more closely related to *E. r. rubecula* than are birds from Gran Canaria. Statistical analysis of measurements also showed significant differences in wingtip shape. We propose to treat the robins from the Canary Islands as a superspecies containing *E. [r.] rubecula* (western Canary Islands and Europe), *E. [r.] superbus* (Tenerife) and *E. [r.] canariae* (Gran Canaria).
Key Words: Phylogeographic differentiation, mitochondrial cytochrome-b sequences, Canary Islands, *Erithacus rubecula*, morphometrics.

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The Canarian archipelago (Spain) consists of seven major islands in the eastern Atlantic Ocean (between 27° 37' and 29° 25' N, and 13° 20' and 18° 10' W), the distance to adjacent African mainland being between 110 km (Fuerteventura) and 460 km (La Palma). The islands are of volcanic origin and, according to general opinion, have never been connected to the African continent (Abdel-Monem et al. 1971, Kunkel 1976, Schmincke 1979, 2000). The age of the islands increases from east to west and ranges from less than 1 million to 20 million years (Fig. 1). Together with Madeira, the Azores and Cape Verde Islands, the Canary Islands form the Macronesian archipelago. Because of these characteristics the Canary Islands are a prime lo-

cation for investigations into the evolution and development of oceanic island biota. This is documented in an increasing number of publications with a focus on molecular phylogeny (e.g. Estoup et al. 1996, Gonzalez et al. 1996) and phylogeography (e.g. Thorpe et al. 1994, Brown & Pestano 1998, Emerson et al. 1999, Nogales et al. 1998) of different animal taxa. These papers highlight the differences in the colonisation pathways and histories of mainly flightless beetle species and reptiles (for review see Juan et al. 2000).

Due to their oceanic origin and volcanic history the Canarian flora and fauna contain a large proportion of endemic species, comparable to the Hawaiian and Galapagos Islands. The percentage of endemism in the
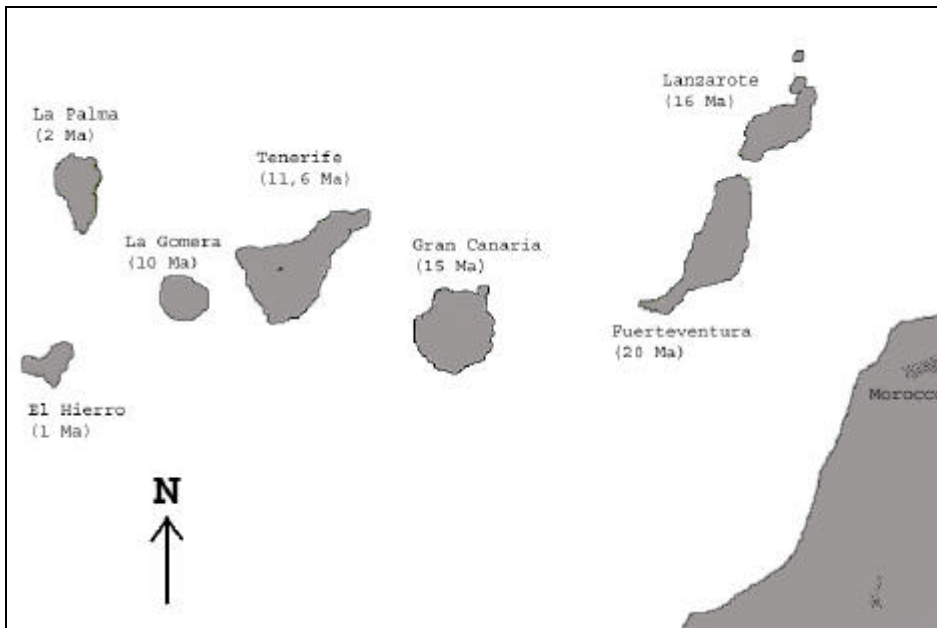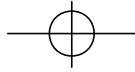
Figure 1. Location and age of the Canary Islands, Spain (after Juan et al. 2000). Ma = million years

native flora is approximately 30 % but can reach more than 90 % depending on the vegetational zone investigated (Schönfelder & Schönfelder 1997, Bergmann 2000). Animal taxa achieve similar values to plants, for example 70 % of carabid beetles, 42 % of aculeate hymenopterans (Bergmann 2000) and 100 % of reptiles (Bischoff 2000) are endemic. Amongst the 75 native breeding birds seven species (including two extinct) are endemic to the Canaries (13 %) and a further three are Macronesian endemics (Bannermann 1963, Martin & Lorenzo 2001). Most of the remaining species are represented by endemic subspecies (n = 26; 35 %) distinct from their closest relatives on the European and African mainland (Baez 1992, Martin & Lorenzo 2001).

Our current knowledge of the taxonomy and systematic position of the Canarian avifauna is mainly based on morphological and bioacoustical studies (e.g. Vaurie 1959, 1965, Cramp 1988). So far, the systematics of only a few Canarian bird taxa have been investigated by using molecular tools, e.g. pipits *Anthus* spp. (Arctander et al. 1996), chaffinches *Fringilla* spp. (Marshall & Baker 1999), stonechats *Saxicola* spp. (Wittmann et al. 1995, Wink et al. 2002a, b), chiffchaffs *Phylloscopus* spp. (Helbig et al. 1996), and bustards *Chlamydotis* spp. (Gaucher et al. 1996, Broders et al. 2003). The use of molecular genetics for answering phylogenetic questions has become a valuable and widely applied tool,

especially if morphologically similar and closely related taxa are involved (e.g. Helbig et al. 1996, Wink et al. 1993, Heidrich & Wink 1994, Helbig & Seibold 1999).

The European robin *Erithacus rubecula* is distributed over large parts of the Western Palaearctic from western Siberia in the east to the Iberian Peninsula in the west (Cramp 1988). Several subspecies have been described (Vaurie 1955, 1959, Cramp 1988, Pätzold 1995) but the morphological differences are merely clinal and not very distinct. The nominate form *E. r. rubecula* inhabits large parts of Europe and northwest Africa and the western Canary Islands (La Gomera, El Hierro, La Palma), Madeira, and the Azores. The birds from these Atlantic islands have formerly been regarded as a separate subspecies *E. r. microrhynchos* (e.g. Hounsome 1993, Martin & Lorenzo 2001) but are usually included in *rubecula* (Lack 1946, 1951, Vaurie 1959, Cramp 1988, Clements 2000). The subspecies *E. r. melophilus* from the British Isles shows a slightly more intensive breast colouration and more olive upperparts. *E. r. witherby* from northern Africa is similar to *melophilus*. Several other subspecies occurring in eastern Europe, the Balkans and the Middle East are almost indistinguishable from the nominate form. The most obvious taxon, *E. r. superbus*, which inhabits the mountain forests of Tenerife and Gran Canaria, is easily separated from the nominate
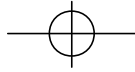
form by its deep orange-red breastpatch, white eye ring, grey forehead and necksides, and white belly (Koenig 1890, Vaurie 1959, Cramp 1988). Recent morphological and acoustical research led to proposals for specific recognition of this taxon as *E. superbus*, the 'Tenerife robin' (e.g. Bergmann & Schottler 2001). Due to the lack of suitable habitat the two desert islands of Fuerteventura and Lanzarote are not inhabited by robins and the species there occurs in small numbers only during migration (Martin & Lorenzo 2001).

A project on the molecular phylogeography of several passerine bird species in the Macronesian Archipelago gave us the opportunity to investigate the systematics of *Erithacus rubecula* on the Canary Islands by using molecular tools. We used sequences of the mitochondrial cytochrome b-gene to study the phylogeographic differentiation, and test the phylogenetic relationships of the taxa involved, in particular the validity of the specific status of *E. superbus* as proposed by Bergmann & Schottler (2001). A further objective concerned the colonisation history of the robin in the Macronesian Archipelago.

Material and methods

Samples

The samples for this study were obtained from live birds on the Canary Islands in 2002 (Table 1). The birds were captured with mist-nets, measured, weighed and small blood samples obtained by puncturing the brachial vein. Afterwards the birds were released and the blood samples preserved in storage buffer containing 0.1 M Tris, pH 7.4, 10 % EDTA, 1 % NaF, 0.1 % thy-
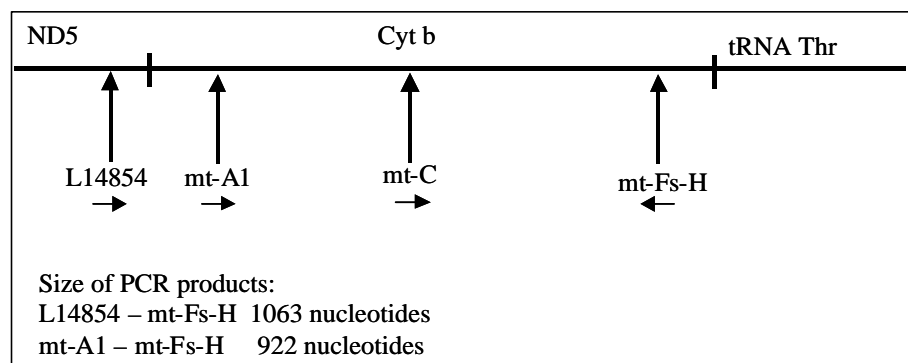
mol and frozen at –20 °C as soon as possible until further processing. Blood samples were collected with permission of the Consejería de Política Territorial y Medio Ambiente (permit No 249).
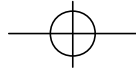
Sequencing

Total genomic DNA was extracted from the stored blood samples by an overnight incubation at 37 °C in lysis buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 75 mM NaCl, 1 % SDS) including 1 mg Proteinase K (Boehringer Mannheim) followed by a standard phenol/chloroform protein extraction. DNA was precipitated from the supernatant with 0.8 volumes of cold isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

The mitochondrial cytochrome b-gene was amplified by PCR from the total genomic DNA using the specific primers L14854 (5'-GGK TCT TTC GCC CTM TC-3'), mt-A1 (L14995; 5'-GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC TTC CG-3') with mt-Fs-H (H15917; 5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3'; cf. Fig. 2). 'K' is coding for guanosine or thymidine, 'M' for adenosine or cytidine and 'Y' for thymidine or cytidine. The total reaction volume was 50 µl containing 1.5 mM MgCl, 10 mM Tris (ph = 8.5), 50 mM KCl, 100 μM dNTPs, 0.8 units Taq polymerase (Pharmacia Biotech, Freiburg), 200 ng DNA and 5 pmoles of the above primers. The cycle protocol consisted of (1) an initial denaturation at 94 °C for 10 min, (2) 30 cycles including denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min and extension at 72 °C for 2 min followed by (3) a final extension period at 72 °C for 10 min. PCR products were stored at 4 °C until further processing. Before

Figure 2. Position of primer sequences used for PCR and sequencing reactions in the mitochondrial genome of European robins and size of PCR products with different primer combinations. Small arrows indicate forward (→) and reverse (←) primers.

ND5   Cyt b   tRNA Thr

L14854   mt-A1   mt-C   mt-Fs-H

Size of PCR products:
L14854 – mt-Fs-H   1063 nucleotides
mt-A1 – mt-Fs-H   922 nucleotides

Table 1. Sampling locations for European robins *Erithacus rubecula* examined in this study.

No	Location	Latitude/Longitude	Island
R01	Maraditas	28° 42' N 17° 48' W	La Palma
R02	Laguna de Barlovento	28° 48' N 17° 48' W	La Palma
R03	Roque Nublo	27° 56' N 15° 36' W	Gran Canaria
R04	Roque Nublo	27° 56' N 15° 36' W	Gran Canaria
R05	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R06	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R07	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R08	El Portillo	28° 18' N 16° 33' W	Tenerife
R10	Frielendorf	50° 58' N 09° 20' E	Germany
R11	Ladera de Tigaiga	28° 20' N 16° 31' W	Tenerife
R12	Ladera de Tigaiga	28° 20' N 16° 31' W	Tenerife
R15	Ladera de Tigaiga	28° 20' N 16° 31' W	Tenerife
R16	Ladera de Tigaiga	28° 20' N 16° 31' W	Tenerife
R17	Monte del Aguas	28° 19' N 16° 49' W	Tenerife
R18	Monte del Aguas	28° 19' N 16° 49' W	Tenerife
R19	Batán de Arriba	28° 31' N 16° 18' W	Tenerife
R20	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R21	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R22	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R23	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R24	Aguagarcia/Lomo de la Jara	28° 27' N 16° 22' W	Tenerife
R25	Barranco de Fernando	28° 49' N 17° 57' W	LaPalma
R26	Lomo de los Pajaros	28° 41' N 17° 47' W	LaPalma
R27	Juego de Bolas (Las Rosas)	28° 10' N 17° 12' W	La Gomera
R28	Lomo de la Mulata	28° 04' N 17° 15' W	La Gomera
R29	Lomo de la Mulata 2	28° 04' N 17° 15' W	La Gomera
R30	Hormiga	28° 09' N 17° 13' W	La Gomera
R31	Cruz de los Reyes	27° 42' N 18° 01' W	El Hierro
R32	Raya la Llania	27° 44' N 18° 00' W	El Hierro
R33	San Salvador	27° 43' N 18° 01' W	El Hierro
R34	La Mareta	27° 46' N 17° 59' W	El Hierro
R35	Reserva Natural de El Brezal	28° 07' N 15° 37' W	Gran Canaria
R36	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R37	Barranco del Laurel	28° 04' N 15° 35' W	Gran Canaria
R38	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R39	Barranco de la Torre	28° 19' N 13° 54' W	Fuerteventura
R40	Catalina Garcia	28° 16' N 14° 01' W	Fuerteventura
R41	Rio Samora	38° 59' N 08° 52' W	Portugal
R42	Taboaco	41° 06' N 07° 43' W	Portugal
R43	Rio Tedo	41° 06' N 07° 45' W	Portugal
R44	Rio Tedo	41° 06' N 07° 45' W	Portugal
R45	Cruz de los Reyes	27° 42' N 18° 01' W	El Hierro
R46	Cruz de los Reyes	27° 42' N 18° 01' W	El Hierro
R47	Cruz de los Reyes	27° 42' N 18° 01' W	El Hierro
R48	Cruz de los Reyes	27° 42' N 18° 01' W	El Hierro
R49	La Mareta	27° 46' N 17° 59' W	El Hierro
R50	La Mareta	27° 46' N 17° 59' W	El Hierro
R51	La Mareta	27° 46' N 17° 59' W	El Hierro
R52	El Brezal	27° 43' N 18° 00' W	El Hierro
R53	El Brezal	27° 43' N 18° 00' W	El Hierro
R54	Lomo de la Mulata	28° 04' N 17° 15' W	La Gomera
R55	Lomo de la Mulata 2	28° 04' N 17° 15' W	La Gomera
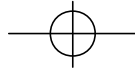
No	Location	Latitude/Longitude	Island
R56	Lomo de la Mulata 2	28° 04' N 17° 15' W	La Gomera
R57	Monte Garajonay	28° 04' N 17° 15' W	La Gomera
R58	Monte Garajonay	28° 04' N 17° 15' W	La Gomera
R59	Reserva Natural de El Brezal	28° 07' N 15° 37' W	Gran Canaria
R60	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R61	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R62	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R63	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R64	Barranco de la Virgen	28° 03' N 15° 34' W	Gran Canaria
R65	Barranco del Laurel	28° 04' N 15° 35' W	Gran Canaria
1.4338	Jan Festo	43° 20' N 05° 22' E	France
3.4340	Heidelberg	49° 24' N 08° 41' E	Germany
4.4341	Madeira	32° 44' N 16° 59' W	Portugal

sequencing PCR products (1 volume) were precipitated with 4 M NH_4Ac (1 volume) and 6 volumes ethanol. After centrifugation for 15 min at 13 000 rpm, DNA pellets were washed in 70 % ethanol and diluted in 15 μl of distilled water.

A cycle sequencing reaction (total volume of 10 μl) contained 2 μl of reaction mix (according to the BigDye Terminator Protocol: Applied Biosystems), 10 pmol primer L14854, mt-A1 or mt-C (L15320; 5'-TAY GTC CTA CCA TGA GGA CAA ATA TCA TTC TGA GG-3'), and 2–5 μl of the template. The cycle sequencing protocol included 25 cycles of 10 s at 96 °C, 5 s at 52 °C and 4 min at 60 °C. Sequencing products were purified by precipitation: 1 volume of reaction mix, 1/10 volumes of 3 M NaAcetate (pH 4.6), 2.5 volumes of ethanol. After centrifugation for 15 min at 13 000 rpm, DNA pellets were washed in 70 % ethanol and diluted in 20 μl of distilled water. The purified sample was diluted 1:5 in water and applied to a 16-column automatic capillary sequencer (ABI 3100) using 50-cm and 80-cm capillaries and POP6 as a polymer. Sequences of other turdid taxa used for comparison were obtained earlier using an ALFexpress II, as described previously (e.g. Wink et al. 2002a).

The sequences used in this analysis are deposited at GenBank under accession numbers AY286333–AY286400.

Phylogenetic Analysis

By using different primer combinations, overlapping sequences with a combined length of 1125 nucleotides were obtained. Sequences were carefully aligned and net pairwise genetic p-distances and corrected Kimura (1980) 2-parameter distances calculated with MEGA version 2.1 (Kumar et al. 2001). Phylogenetic trees were constructed employing PAUP*4b10 (neighbour-joining and maximum parsimony; Swofford 2001) and MrBayes version 2.01 (maximum likelihood; Huelsenbeck & Ronquist 2001). Neighbour-joining analysis was performed using Kimura's (1980) two-parameter model and bootstrapped 1000 times. Results were similar to the maximum parsimony analysis, and only the latter is shown. For maximum parsimony analysis (heuristic search) all characters were unordered and of equal weight. Starting trees were obtained via stepwise addition with addition sequence as closest, and the branch-swapping algorithm was set to tree-bisection-reconnection (TBR). From the resulting 500 shortest trees a strict consensus and a 50 % majority rule consensus tree were estimated. For bootstrap analysis 500 replicates with branch-and-bound algorithm were run. To describe the trees obtained the following statistics were calculated as described by Swofford (2001): tree length, consistency index (CI), homoplasy index (HI), retention index (RI) and rescaled consistency index (RC). For maximum likelihood analysis the Bayesian inference of phylogeny was used (Huelsenbeck & Ronquist 2001). The calculations were based on the general time reversible (GTR) model (Tavaré 1986, Swofford et al. 1996) and performed with 500 000 Markov chains Monte Carlo from a random starting tree. The first 500 trees were ignored. Nucleotide frequencies for the starting tree were estimated (A = 0.27789, C = 0.35630, G = 0.13190, T = 0.23391).
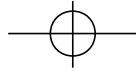
The following population analyses were performed with Arlequin version 2.000 (Schneider et al. 2000). Gene flow between populations was estimated using F-statistics (Wright 1928) and Fst values were interpreted as suggested by Wright (1978). For investigations of population history, pairwise mismatch distributions were calculated after the 'infinite sites' model (Kimura 1971) and plotted against expected values following the 'model of sudden expansion' (Rogers & Harpending 1992). Genetic structure was evaluated using analysis of molecular variance (AMOVA). Two assumed genetic structures were tested with samples from Gran Canaria and Tenerife in one group opposed to the remaining samples in the second group, and with Gran Canaria, Tenerife and the remaining samples each forming separate groups.

Morphometrics

All birds captured for sampling were measured and weighed. The following measurements were taken as described in Svensson (1992): maximum wing length, length of primaries (P) 1–9 and secondary (S) 1, length of tarsus, length of bill tip to distal end of nostril (NaLoSpi), bill width, bill height, bill length from tip to skull and length of footspan for outer, middle and inner toe. Measurements were exact to 0.5 mm (wing) and 0.1 mm (leg and bill) respectively; the weight of the birds was measured using a digital balance (Ohaus CS200) exact to 0.1 g.

All measurements were analysed for variance by MANOVA using SPSS version 5.0.2 (SPSS Inc. 1993). Significance levels were set at $P \leq 0.05$ (* significant) and $P \leq 0.01$ (** highly significant). To investigate possible morphological differentiation between popula-

tions the data were entered into a discriminant function analysis (Wilks's Lambda). Wingtip shape characteristics were calculated following Lockwood et al. (1998). Only adult birds not in moult were included.

Results

The cytochrome b-gene was sequenced from 66 robins and a further seven turdid species of the genera *Turdus* (outgroup), *Luscinia*, and *Saxicola*. The sequences obtained could be aligned without difficulty and no stop codons were encountered. The employment of different primers which produced overlapping sequences gave some additional proof that the sequences were correct and of mitochondrial origin.

1125 nucleotides in the robin dataset showed 226 (20.1 %) variable sites of which 85 (7.5 %) were parsimony informative. The net pairwise genetic p-distances between and within the island populations are shown in Table 2. The distances between *E. r. rubecula* (*rubecula* hereafter) of the western Canary Islands and European mainland and *E. r. superbus* (*superbus* hereafter) varied between 2.7 and 5.1 % (mean 3.8 %). The most striking feature, however, is that *superbus* from Gran Canaria clearly differs from those of Tenerife by 3.7 ± 0.7 %. The *superbus* from Tenerife differ from *rubecula* by 2.7–3.2 % (mean 2.9 %) while a genetic distance of 4.6–5.1 % (mean 4.8 %) was found between *superbus* from Gran Canaria and *rubecula*. In *rubecula* the divergence between different islands including mainland Europe did not exceed 1.1 % (0.11–1.1 %, mean 0.6 %). Within the island populations the genetic distances were small (mean 0.5 %), and the greatest within-group distance was found on Tenerife (1.1 ± 0.2 %).

Table 2. Uncorrected genetic p-distances (below diagonal) and Kimura-2-parameter distances (above diagonal) between populations of European robins *Erithacus rubecula* inferred from 1125 nucleotides of the mitochondrial cytochrome b-gene. In the diagonal (bold) are the within-group distances. Shown are the mean net distances [%] ± s. e.

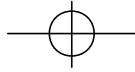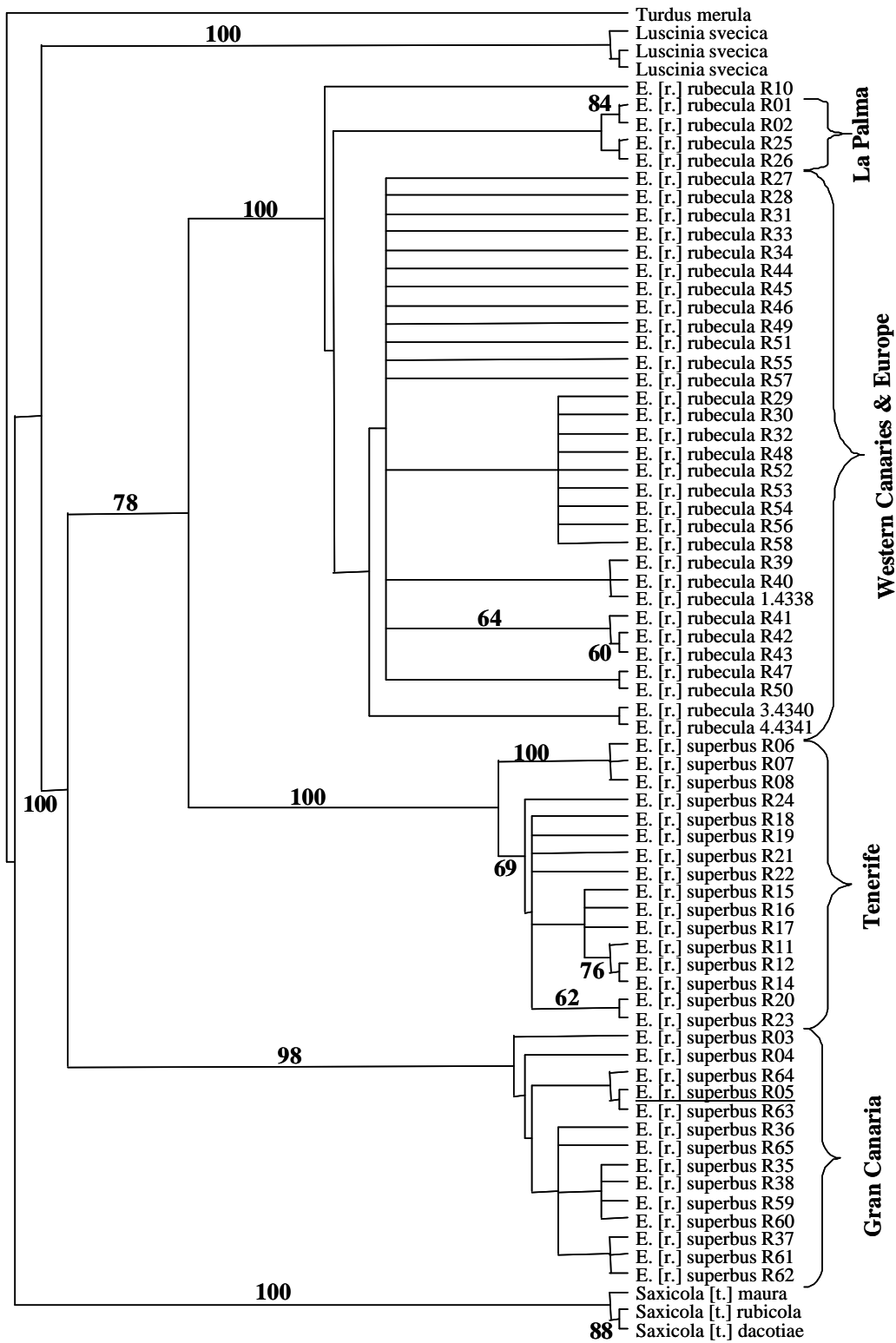|	[1]	[2]	[3]	[4]	[5]	[6]
[1] La Palma	**0.6 ± 0.2**	0.3 ± 0.2	0.4 ± 0.2	3.4 ± 0.7	5.4 ± 1.0	0.5 ± 0.2
[2] La Gomera	0.3 ± 0.2	**0.1 ± 0.1**	0.0 ± 0.0	2.9 ± 0.7	4.9 ± 1.0	0.1 ± 0.1
[3] El Hierro	0.4 ± 0.2	0.0 ± 0.0	**0.1 ± 0.1**	2.8 ± 0.7	4.0 ± 1.0	0.1 ± 0.0
[4] Tenerife	3.2 ± 0.7	2.8 ± 0.6	2.8 ± 0.6	**1.1 ± 0.2**	3.9 ± 0.7	2.8 ± 0.7
[5] Gran Canaria	5.1 ± 0.9	4.7 ± 0.8	4.7 ± 0.8	3.7 ± 0.7	**0.4 ± 0.1**	4.9 ± 0.9
[6] Europe	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	2.7 ± 0.6	4.6 ± 0.8	**0.6 ± 0.2**

Figure 3. Maximum parsimony analysis (50 % majority rule consensus tree) of robin taxa. Numbers refer to bootstrap values above 60 % (500 replicates). Tree length 513, CI = 0.7271, HI = 0.2729, RI = 0.9176, RC = 0.6672. The underlined individual was caught on Tenerife.
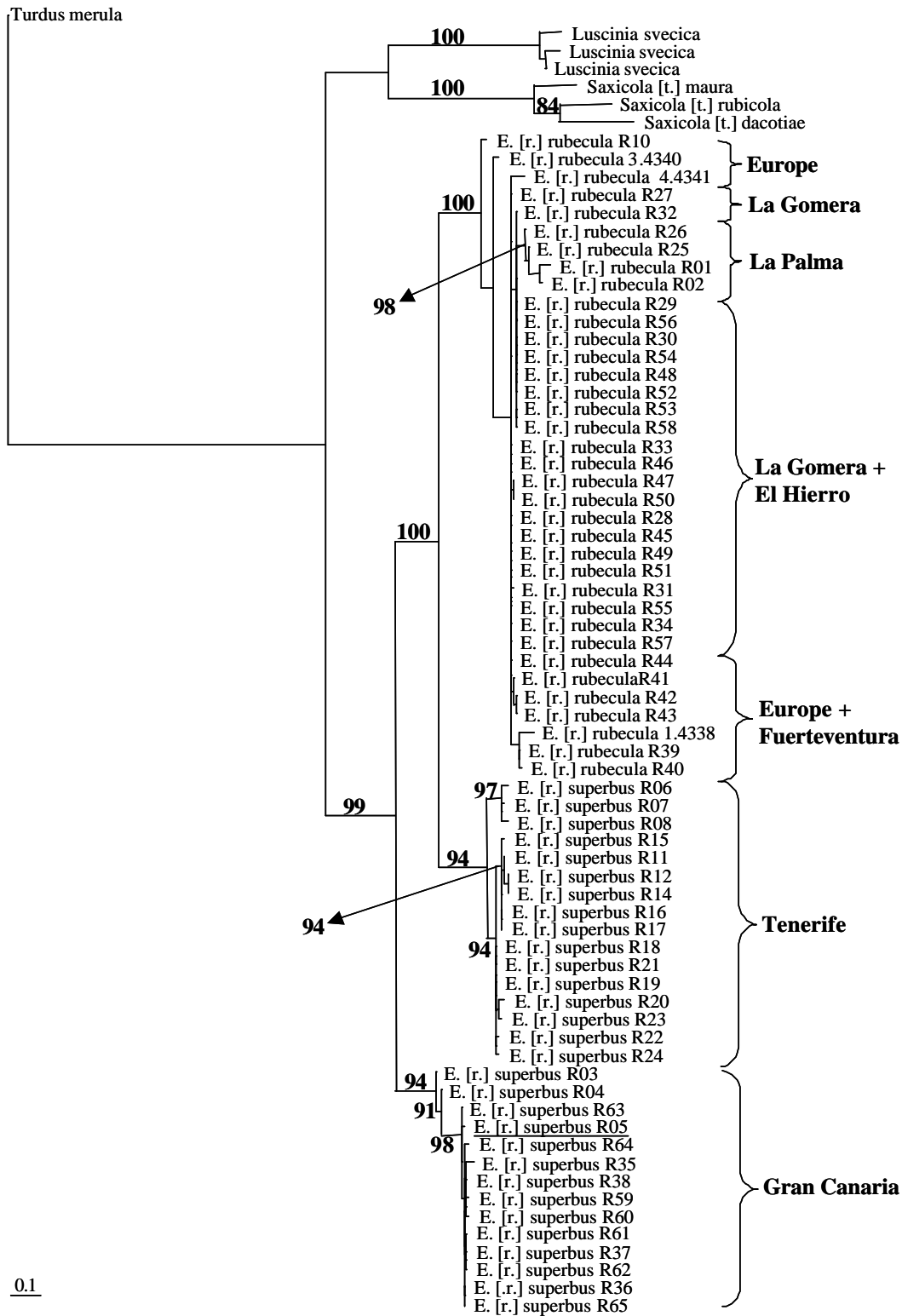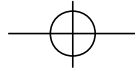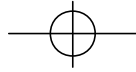
Figure 4. Maximum likelihood analysis following Bayesian inference of phylogeny of robin taxa. Branch lengths correspond to genetic distances. The numbers indicate clade credibility values above 80. The underlined individual was caught on Tenerife.

Most differences within the island populations were due to single nucleotide substitutions. Only on Tenerife could several distinct haplotypes be identified; one bird (sample R05) caught on Tenerife showed strong affinities to the haplotype found on Gran Canaria.

The phylogenetic analysis led to more or less identical tree topologies for all three tree building methods used (see Figs 3 and 4; neighbour joining results are not shown because they show a similar outcome to maximum parsimony and maximum likelihood). The genus *Erithacus* forms a monophyletic clade supported by high bootstrap values (99–100 %) in neighbour-joining and maximum parsimony analyses. Within *Erithacus* three distinct groupings can be recognised. The *superbus* from Gran Canaria take a more basal position and are opposed to a clade comprised of *superbus* from Te-

nerife and all *rubecula*. In this latter clade *superbus* is clearly separated from *rubecula*. All these groupings gain high bootstrap support (81–100 %). According to these results *E. r. superbus* is clearly paraphyletic. In the *rubecula*-clade no stable groupings could be detected with the exception of the birds from La Palma, which usually clustered together (61 % bootstrap support). Some of the Central European birds form a small well supported (82 % bootstrap) cluster within *rubecula*. Also the migrant birds caught on Fuerteventura are included in this cluster. The terminal positions within the groupings could not be resolved satisfactorily from the cytochrome-b sequences and bootstrap values are very low (2–56 %).

Fst values between robin populations from Gran Canaria, Tenerife and the western Canary Islands plus Eu-
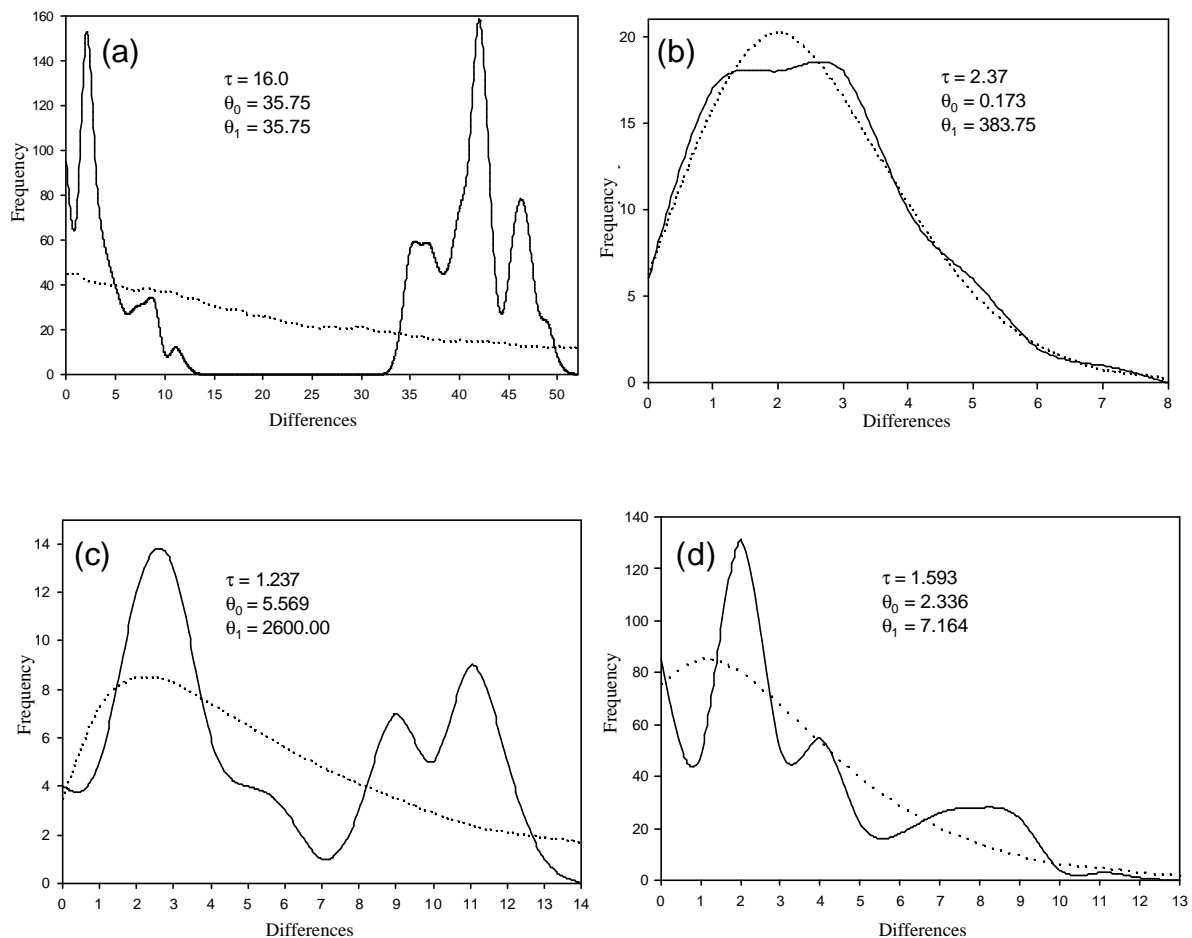

Figure 5. Pairwise mismatch distributions among (a) all individuals of European robins *Erithacus rubecula*, (b) on Gran Canaria, (c) Tenerife and (d) nominate *E. r. rubecula*. Solid lines show the observed distribution and dotted lines the expected distribution after the 'sudden expansion' model (Rogers & Harpending 1992).
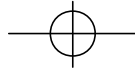
Table 3. Fst values for three populations of European robins *Erithacus rubecula* on the Canary Islands. Significant values are indicated by * P < 0.05, ** P < 0.01 and *** P < 0.001.

Populations compared	Fst
Gran Canaria v. Tenerife	0.9105***
Gran Canaria v. Western Canaries/Europe	0.9278***
Tenerife v. Western Canaries/Europe	0.8960***

rope are all highly significant (Table 3) and indicate a very restricted gene flow between these populations (Wright 1978). But we note that one bird caught on Tenerife is genetically more closely related to the birds from Gran Canaria (cf. Figs 3 and 4). Results of the

AMOVA (not shown) gave much more support to the assumption of three groups (Tenerife, Gran Canaria and *rubecula*), which explains 89.79 % of the total variance, while the classical division into two groups (*superbus* and *rubecula*) could only explain 52.39 % of the total variance.

The pairwise mismatch distribution among all individuals of the genus *Erithacus* is clearly multimodal (Fig. 5a), indicating two classes of comparisons, within and between taxa. For the birds from Gran Canaria the pairwise mismatch distribution shows a relatively smooth and unimodal curve, as is typical for a recent range expansion (Fig. 5b; cf. Rogers 1995). The mismatch distribution for the birds from Tenerife is multimodal indicating geographic structure or population bottlenecks (Fig. 5c), but sample sizes from different

Table 4. Morphometric measurements of European robins *Erithacus rubecula* from the Canary Islands. Significance of variances (F) revealed by MANOVA are marked with n.s. (not significant), * (P < 0.05) or ** (P < 0.01).

| Character	La Palma mean	s.d.	n	La Gomera mean	s.d.	n	El Hierro mean	s.d.	n	Tenerife mean	s.d.	n	Gran Canaria mean	s.d.	n	Fuerteventura mean	s.d.	n	Sign. F
Weight [g]	15.7	0.3	3	15.7	1.1	9	15.9	0.9	13	15.2	2.7	16	15.3	0.9	11	14.8	0.1	2	**
Wing [mm]	72.5	1.5	3	71.9	2.7	9	71.1	2.6	13	68.9	4.8	16	67.5	2.2	11	71.3	0.4	2	**
P9 [mm]	45.3	1.0	3	44.8	1.5	9	45.5	2.0	12	43.3	0.4	2	41.2	1.7	10	44.3	1.1	2	**
P8 [mm]	54.5	0.5	3	53.6	2.1	9	53.8	2.1	12	52.3	0.4	2	49.5	1.5	10	54.0	1.4	2	**
P7 [mm]	57.7	0.3	3	56.9	2.1	9	57.0	1.9	12	56.0	1.4	2	53.2	1.6	10	57.3	0.4	2	**
P6 [mm]	59.3	0.6	3	58.7	2.5	9	58.0	2.1	12	58.3	2.5	2	55.0	2.0	10	58.3	0.4	2	**
P5 [mm]	59.2	0.8	3	59.2	2.2	9	58.0	1.8	12	58.8	1.8	2	55.7	2.1	10	58.3	1.1	2	*
P4 [mm]	55.2	0.3	3	55.4	1.7	9	54.7	1.9	12	57.5	1.5	2	53.3	1.9	10	54.3	1.8	2	*
P3 [mm]	53.2	0.8	3	53.7	1.6	9	52.6	1.6	12	55.0	1.4	2	51.9	1.8	10	52.8	1.1	2	n.s.
P2 [mm]	52.3	0.6	3	52.8	1.8	9	51.4	1.6	12	54.0	1.4	2	50.7	1.6	9	52.0	1.4	2	n.s.
P1 [mm]	52.3	1.0	3	52.2	1.9	9	50.9	1.7	12	53.5	0.7	2	50.1	1.5	10	51.8	1.8	2	n.s.
S1 [mm]	51.2	0.3	3	51.9	2.0	9	50.5	1.7	12	53.0	1.4	2	49.7	1.6	10	51.0	1.4	2	n.s.
Tarsus [mm]	24.4	0.8	3	24.1	0.8	9	24.4	0.9	12	24.0	0.1	2	25.3	1.0	10	24.6	0.8	2	n.s.
NaLoSpi [mm]	7.3	0.4	2	7.1	0.5	9	7.3	0.4	12	7.2	0.2	2	7.1	0.6	9	6.9	0.3	2	n.s.
Bill width [mm]	4.7	0.4	2	4.7	0.3	9	4.7	0.4	12	4.4	0.1	2	4.8	0.3	9	4.3	0.3	2	n.s.
Bill length [mm]	15.2	0.8	3	15.4	0.6	9	15.8	0.4	12	16.1	0.3	2	16.0	0.5	10	15.2	0.5	2	n.s.
Bill height [mm]	3.2	0.0	2	3.2	0.1	9	3.4	0.2	12	3.3	0.2	2	3.3	0.3	9	3.4	0.1	2	n.s.
Foot in [mm]	26.0	1.4	2	25.8	0.8	9	26.0	1.0	12	26.0	0.0	2	26.0	0.8	8	24.3	0.4	2	n.s.
Foot mid [mm]	32.5	2.1	2	32.6	1.0	9	33.0	1.3	12	33.0	1.4	2	32.3	1.3	8	30.5	0.7	2	n.s.
Foot out [mm]	27.0	1.4	2	26.4	0.9	9	27.1	0.8	12	27.0	0.0	2	26.9	0.6	8	25.5		1	n.s.
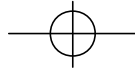
Table 5. Results of the discriminant function analysis of measurements from European robins *Erithacus rubecula* on the Canary Islands. Canonical discriminant function coefficients (lines 1–3) are shown for the characters entered in the analysis (primaries (P) 4 + 8 and tarsus length).

	Function 1	Function 2	Function 3
P4	0.82071	1.19131	0.34852
P8	−1.56119	−0.49286	0.18676
Tarsus	0.82694	−0.35239	0.74861
Eigenvalues	2.3904	0.3584	0.0012
Percent variation	86.92 %	13.03 %	0.04 %
Cumulative percentage	86.92 %	99.96 %	100 %
Canonical correlation	0.8397	0.5136	0.0349

parts of the island are too small to distinguish between these options. The mismatch distribution for the nominate form is rather ragged (Fig. 5d) as is usually shown in populations in equilibrium (Zink 1997).

Statistical analysis of morphological measurements shows significant variance between populations, mainly due to differences in primary length and wingtip shape (Table 4). Average wing length increases obviously from Gran Canaria via Tenerife to the other islands but there is some overlap (Table 4). The mean length of P9 to P1 is shorter in birds on Gran Canaria than in those of Tenerife (Fig. 6). There is an obvious difference in the wing shape between birds from Gran Canaria and Tenerife as compared to those of the other islands. The former have a more rounded and convex

wing than the latter (Figs 6 and 7). The discriminant function analysis shows that the birds from the European mainland and the western Canary Islands are not separable but birds from Gran Canaria and Tenerife are different from each other and *rubecula*, respectively (Fig. 8). The analysis yielded three functions which explain 100 % of the variance between populations (Table 5).

Discussion

In the past, robins inhabiting the Canary Islands have been assigned to two subspecies. The birds on the westernmost islands (La Palma, El Hierro and La Gomera) were thought to belong to the nominate form *E. r. rube-*
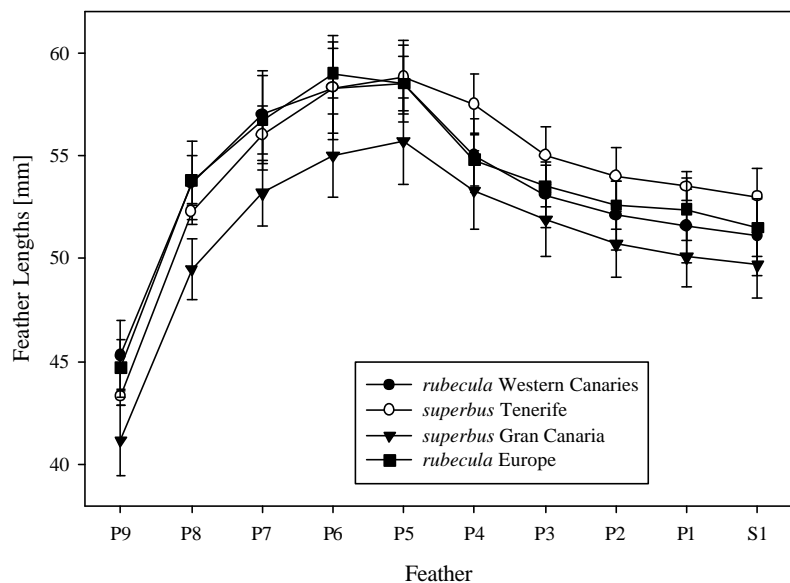

Figure 6. Wing shape of European robins *Erithacus rubecula* on different Canary Islands and Europe (Portugal) based on measurements of primaries (P) 1–9 and secondary (S) 1.
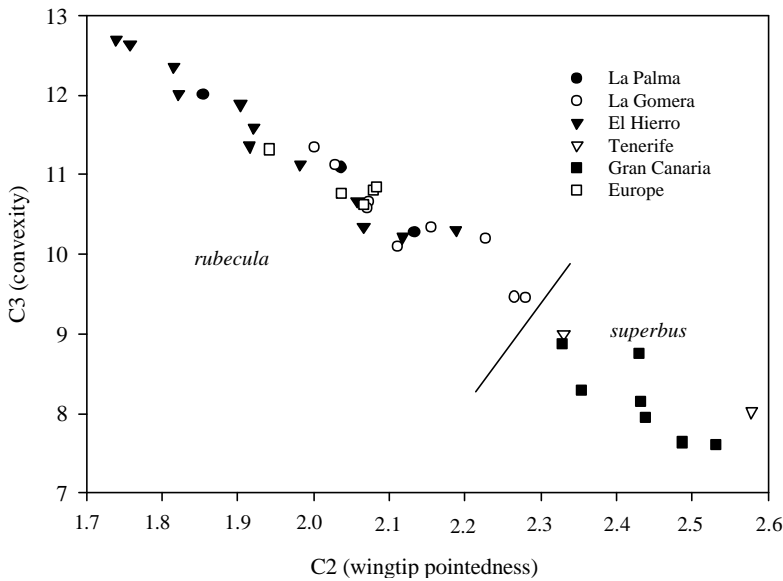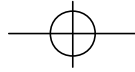
Figure 7. Wingtip shape of European robins *Erithacus rubecula* in the Canary Islands. The two indices for characterisation of wing-shape were calculated following Lockwood et al. (1998). A decrease in C2 leads to an increase in pointedness while increasing C3 leads to an increase in convexity.
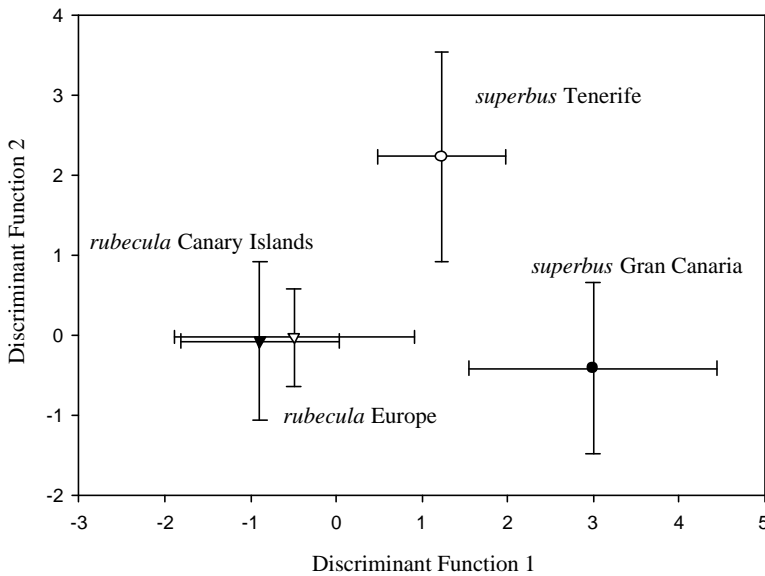

Figure 8. Plot of the first two of three discriminant functions for different populations of European robins *Erithacus rubecula* on the Canary Islands. The group centroids with standard deviations are shown.

cula (e.g. Cramp 1988) or to constitute another Macronesian subspecies together with birds from Madeira, *E. r. microrhynchus* (e.g. Hounsome 1993), while the birds from Gran Canaria and Tenerife were regarded as a subspecies of their own, *E. r. superbus* (Koenig 1890, Vaurie 1959, Cramp 1988). Recent analysis of song structure together with the distinct plumage differences led Bergmann & Schottler (2001) to propose species status for the latter taxon, the Tenerife robin, *E. superbus*.

From the genetic data it is evident that we have to distinguish between *superbus* from Gran Canaria and Tenerife. The former take a more basal position, while the robins from Tenerife are more closely related to *rubecula*. Robins from Gran Canaria and Tenerife show independent genetic histories in the maternally inherited mitochondrial genome and have clear morphometric differences. Assuming a molecular clock of 2 % divergence for one million years (Shields & Wilson 1987) the populations on Gran Canaria and Tenerife have diverged independently from other island or European mainland populations 2.3 and 1.8 million years ago respectively. The degree of divergence between islands
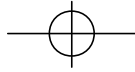
increases with island age. From the genetic data it seems possible that Gran Canaria, the oldest island (15 Ma) of those inhabited by robins today, was colonised first by a common ancestor, followed by independent colonisation of Tenerife (12 Ma) by the common ancestor of the Tenerife robin and *rubecula*, while the western islands (1–10 Ma) were colonised fairly recently (c. 350 000 years ago), probably during Pleistocene glaciations. The strong similarities in colouration suggest that the common ancestor of today's robins was closer in appearance to *superbus*, and that the duller plumage of *rubecula* originated fairly late, after the colonisation of Tenerife and Gran Canaria. Another explanation, which has yet to be tested when samples from northern Africa are available, is whether the Canary Islands were colonised in two waves: the eastern islands of Tenerife and Gran Canaria from Africa and the western islands from Europe. Then Tenerife could form a contact zone between populations derived from Africa and Europe. This would also explain the higher degree of heterozygosity found on Tenerife as compared to the other islands.

Considering the results of the genetic comparisons, it is no longer tenable to regard the robins of Gran Canaria and Tenerife as one taxon (neither species nor subspecies). The pairwise genetic distances between *superbus* from Tenerife and Gran Canaria are as large as those between *rubecula* and *superbus* from either island (see Table 2). With regard to the genetic results (distance data, phylogenetic analysis) three distinct groups can be recognised: (1) *E. r. rubecula* from Europe and the western Canary Islands, (2) *E. r. superbus* from Tenerife, and (3) *E. r. superbus* from Gran Canaria. All these groups show distinct mitochondrial cytochrome-b haplotypes and are separated by large genetic distances. Similar pairwise distances are found between good species of other closely related passerines (see Table 6). The between-group genetic distances exceed the range of 0.2–2.6 % usually assumed for subspecies and fall well within the range of good species with genetic distances of 0.5–3 % and more (Helbig et al. 1995). Although the geographical distances between the islands are small, no notable gene exchange (significant Fst values, cf. Table 3) seems to occur between e.g. Tenerife and La Gomera. Only one bird caught on the northern slope of the Teide mountain, Tenerife, showed close affinities to the haplotype from Gran Canaria, indicating occasional migration between these islands. There are no indications for a substantial gene flow between the eastern islands. The open water between two islands works as a strong isolating barrier preventing exchange between populations.

The examination of the pairwise mismatch distributions (Fig. 5) with respect to the phylogenetic data provides evidence for a single colonisation of Gran Canaria followed by a range expansion on this island. Tenerife or its precursor islands was maybe colonised more than once, resulting in the observed multimodal distribution and the intermediate morphometric characteristics. More samples are needed to verify this hypothesis.

The results from our genetic study are in contrast to published morphological and bioacoustical analyses. In the recent literature there is no indication that *superbus* from Gran Canaria and Tenerife differ in plumage, mor-

Table 6. Pairwise genetic distances for closely related passerine taxa from published cytochrome-b sequence data.

Species-pair	Genetic distance [%]	Source
Sitta krueperi/sedanti	3.5	Pasquet (1998)
Acrocephalus seychellensis/newtoni	4.7	Leisler et al. (1997)
Acrocephalus avicenniae/scirpaceus	2.0	Leisler et al. (1997)
Hippolais icterina/polyglotta	6.5	Helbig & Seibold (1999)
Luscinia luscinia/megarhynchos	6.4	Wink et al. (2002a)
Saxicola rubicola/maura	4.3	Wink et al. (2002a)
Phylloscopus collybita/brehmii	4.2	Helbig et al. (1996)
Phylloscopus collybita/canariensis	3.7	Helbig et al. (1996)
Phylloscopus nitidus/viridanus	3.1	Helbig et al. (1995)
Anthus correndera/antarcticus	2.7	Voelker (1999)
Anthus rubescens/japonicus	3.3	Voelker (1999)
Serinus citrinella/corsicana	2.7	Pasquet & Thibault (1997), Sangster (2000)
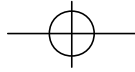
phometrics or acoustics (e.g. Vaurie 1959, Cramp 1988, Martin & Lorenzo 2001, Bergmann & Schottler 2001). However, as far as we are aware, there has been no study concentrating on potential differentiation between robins of Gran Canaria and Tenerife, because all authors assumed these two populations to be conspecific. It seems possible that small differences could exist but have been overlooked due to the assumption that only one taxon is involved. However, statistical analysis of our measurements indicates morphological differences between *superbus* from Tenerife and Gran Canaria, as well as between *rubecula* and both populations of *superbus*. The *superbus* from Tenerife with relatively long primaries and rounded wings are again situated intermediately between *superbus* from Gran Canaria (short and rounded wings) and *rubecula* (long and pointed wings; cf. Figs 6–8). These characters are in line with the so called 'island syndrome' (e.g. shorter, more rounded wings, increased biometric variability, smaller size, wider niche occupation, change from migrant to resident populations; Hounsome 1993) and are of little value for systematic analysis (Helbig et al. 2002). Due to small sample sizes for some island populations, we regard these results as preliminary and in need of further verification with larger sample sizes.
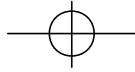
Hounsome (1993) found a clear differentiation between *superbus* and *rubecula*. Furthermore he noted the robins from the western islands to be identical with those from Madeira and both differed from British robins. From these results he concluded the validity of *E. r. microrhynchos* as separate taxon and that Atlantic robins are different from *rubecula*. But since he did not include true *rubecula* in his analysis (British robins belong to *E. r. melophilus*) this conlusion is misleading. The Madeiran robin included here falls well into *rubecula* and there is no evidence for another taxon, i.e. *E. r. microrhynchus,* in the eastern Atlantic islands.

Our cytochrome-b sequence data, as well as the morphological information, give no indication for any obvious differentiation between *rubecula* from the western Canaries and Europe. Following this, we suggest keeping the Canary robins within nominate *rubecula* (cf. Clements 2000, Cramp 1988, Lack 1946). The data presented here indicate a relatively recent colonisation of the western islands which explains the lack of genetic and morphological differentiation. Low Fst values (not shown) indicate some geneflow between these islands since the birds involved are probably still more migratory than those on the eastern islands. It would be premature under any species concept to split *Erithacus* of the Canary Islands into three species as the genetic and part of the morphological data suggest. Following the Evolutionary Species Concept (ESC) we propose to treat the taxa involved as a superspecies (cf. Helbig et al. 2002). The taxa should then be named as (1) *E. [r.] rubecula* (Western Canaries, Europe and probably Azores and Madeira), (2) *E. [r.] superbus* (Tenerife) and (3) *E. [r.] ssp.* (Gran Canaria). This genetic structuring is supported by the analysis of molecular variance.

For conservationists our finding of two distinct taxa on Gran Canaria and Tenerife is quite important. Especially on the former island, the natural habitats are severely degraded and destroyed due to human activities, e.g. deforestation, lowering of groundwater table etc. This has resulted in the extinction of several taxa in the past (Johnson & Stattersfield 1990, Martin et al. 2000, Martin & Lorenzo 2001). On Gran Canaria the remaining forest cover is restricted to very few mountainous regions. The numbers and distribution of robins and other forest-depending species (e.g. Blue chaffinch *Fringilla teydea polatzeki*) are declining (Martin & Lorenzo 2001). This endangered forest bird community certainly needs more attention from politicians and conservationists, especially on the densely populated island of Gran Canaria. This is particularly important when different evolutionary lineages are involved, as seems to be the case with the endemic robin.
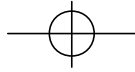
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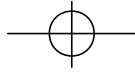
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