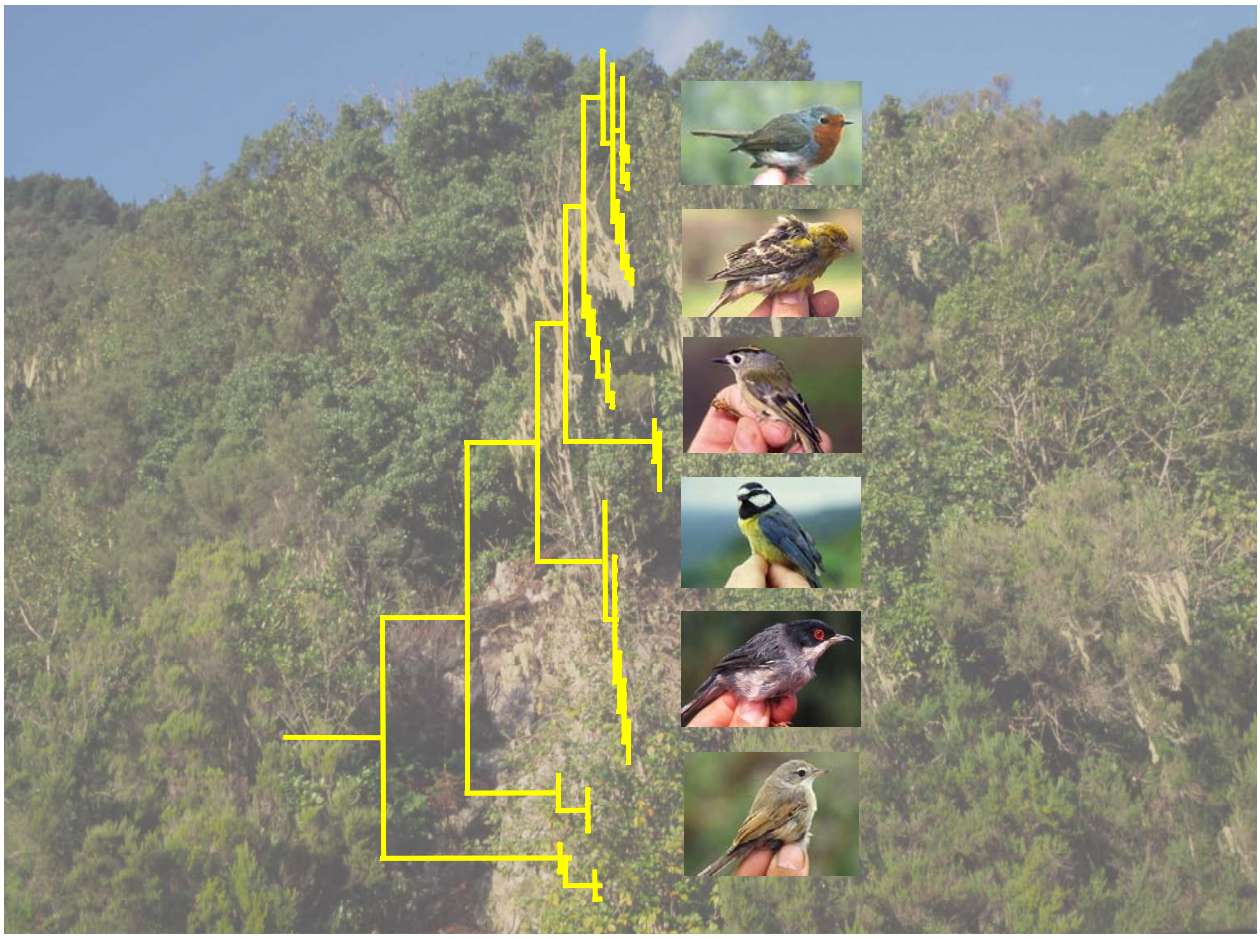
Molecular phylogeography and colonization history of passerine birds of the Atlantic islands (Macaronesia)



Christian Dietzen

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DISSERTATION

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Oral examination:

Molecular phylogeography and colonization history of passerine birds of the Atlantic islands (Macaronesia)

Referees: Prof. Dr. Michael Wink

Prof. Dr. Dr. Volker Storch

"Regarding the small size of these islands the sheer amount of endemic species is really remarkable. Furthermore, every mountain is crowned by a young crater and the borders of each lava flow are still clearly recognisable. We have to conclude that not long ago, the ocean was reigning out here. It seems to me, that here in space as well as in time, the secret of all secrets, that is the appearance of new creatures on earth is readily perceptible."

CHARLES DARWIN, 1845

Publications

DIETZEN, C., WITT, H.-H. & M. WINK (2003): 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. – Avian Science **3**: 115-131

WINK, M., DIETZEN, C. & B. GIEßING (2005): Die Vögel des Rheinlandes (Nordrhein). Ein Atlas zur Brut- und Wintervogelverbreitung 1990 bis 2000. Romneya Verlag, Dossenheim. 419 S.

DIETZEN, C., VOIGT, C., WINK, M., GAHR, M. & S. LEITNER (2006): Phylogeography of island canary (*Serinus canaria*) populations. – Journal of Ornithology **147**: 485-494

PÄCKERT, M., DIETZEN, C., MARTENS, J., WINK, M. & L. KVIST (2006): Radiation of Atlantic goldcrests *Regulus regulus* spp.: evidence of a new taxon from the Canary Islands. – Journal of Avian Biology **37**: 364-380

DIETZEN, C., HACKENBERG, C., HEYNE, K.-H., SAUER-GÜRTH, H., STAUDTER, H. & M. WINK (2007): Genetically confirmed interbreeding between western Bonelli's warbler (*Phylloscopus bonelli*) and wood warbler (*P. sibilatrix*). – Journal of Ornithology **148**: 85-90

PÄCKERT, M., MARTENS, J., TIETZE, D. T., DIETZEN, C., WINK, M. & L. KVIST (2007): Calibration of a molecular clock in tits (Paridae)- nucleotide substitution rates of mitochondrial genes deviate from the 2% rule. – Molecular Phylogenetics and Evolution **44**: 1-14

DIETZEN, C., GARCIA-DEL-REY, E., DELGADO CASTRO, G., & M. WINK (submitted to Journal of Ornithology): Phylogeography of the blue tit (*Parus teneriffae* – group) on the Canary Islands based on mitochondrial DNA sequence data and morphometrics.

DIETZEN, C., GARCIA-DEL-REY, E., DELGADO CASTRO, G., & M. WINK (submitted to Biological Journal of the Linnean Society): Phylogenetic differentiation of the passerine genus *Sylvia* of the Atlantic islands (Macaronesia) based on mitochondrial DNA sequence data and morphometrics

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Summary

Based on mitochondrial cytochrome b sequence data and morphological data the phylogeography and phylogenetic relationships of selected passerine bird species on the Atlantic islands (Macaronesia) of the European robin (*Erithacus rubecula*), island canaries (*Serinus canaria*), goldcrest (*Regulus regulus*), blue tit (*Parus teneriffae*-group) and *Sylvia* warblers (*S. melanocephala, S. atricapilla, S. conspicillata*) were investigated. Very strong genetic differentiation including so far not recognized new taxa were found in the robin, goldcrest and blue tit. In contrast, genetic differentiation was weak in island canaries and *Sylvia* warblers. For most species the genetic data provide evidence for multiple independent colonizations. The most recent colonizations can be correlated with Pleistocene glaciations (0.01 – 2 my ago). Molecular data are, at least partially, supported by morphological and bioacoustic findings.

It is proposed 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 a new taxon *E.* [*r.*] *marionae* nov. sp. from Gran Canaria.

The colonization of the Atlantic islands by the canaries occurred very recently, while there is no persisting gene flow between the populations.

The Azorean goldcrest (*Regulus regulus*) populations are closely related to European nominate *R. r. regulus*. The Canarian populations are genetically substructured into a northeastern group embracing Tenerife and La Gomera and a second, southwestern group including El Hierro and La Palma. As a taxonomic consequence of the marked differentiation of the two Canarian goldcrest clades the populations from El Hierro and La Palma are described as a taxon new to science and are named *Regulus regulus ellenthalerae* n. ssp.

Taxonomic recommendations for the blue tit include the distinction of *P. caeruleus* from northern Europe and *P. teneriffae*, including North Africa and the Canary Islands, the treatment of *degener* and *ultramarinus* as synonymous (*P. teneriffae ultramarinus*) and a new blue tit taxon on the island of Gran Canaria (*P. t. hedwigii* nov. ssp.), which is formally described.

The subspecific distinctiveness for Sardinian warblers (*Sylvia melanocephala*) and blackcaps (*S. atricapilla*) from the Atlantic islands was rejected. Differences in morphometrics in both Sardinian warbler and blackcap are rather caused by migratory behaviour and ecological traits than by phylogeny. Tentative data of a small sample of spectacled warblers (*S. conspicillata*) also suggest a low degree of differentiation.

Zusammenfassung

Die Phylogeographie und phylogenetischen Beziehungen ausgewählter Singvogelarten auf den Atlantischen Inseln (Makaronesien) wurden basierend auf mitochondriellen Cytochrom b Sequenzdaten und der Morphologie von Rotkehlchen (*Erithacus rubecula*), Kanarengirlitz (*Serinus canaria*), Wintergoldhähnchen (*Regulus regulus*), Blaumeise (*Parus teneriffae*-group) und Grasmücken (*S. melanocephala, S. atricapilla, S. conspicillata*) untersucht. Sehr starke genetische Differenzierungen einschließlich bisher unbeschriebener neuer Taxa wurden bei Rotkehlchen, Goldhähnchen und Blaumeise gefunden. Im Gegensatz dazu war die genetische Differenzierung bei Kanarienvögeln und Grasmücken sehr schwach. Die genetischen Daten suggerieren für die meisten Arten mehrere unabhängige Besiedlungen. Die rezentesten Besiedlungen lassen sich mit der letzten Eiszeit vor 0.01 – 2 mio. Jahren (Pleistozän) korrelieren. Die molekularen Daten wurden zumindest teilweise von morphologischen und bioakkustischen Daten unterstützt.

Die Rotkehlchen der Kanarischen Inseln sollten in einer Superspezies aus *E.* [*r.*] *rubecula* (westl. Kanaren und Europa), *E.* [*r.*] *superbus* (Teneriffa) und einem neuen Taxon *E.* [*r.*] *marionae* nov. sp. von Gran Canaria behandelt werden.

Die Kolonisierung der Atlantischen Inseln durch Girlitze erfolgte sehr rezent, während es keine Anzeichen für anhaltenden Genfluss zwischen den einzelnen Populationen gibt.

Die Goldhähnchen von den Azoren sind nahe mit der europäischen Nominatform *R. r. regulus* verwandt. Die Kanarischen Goldhähnchen zeigen dagegen eine deutlich Differenzierung in eine nordöstliche Gruppe auf Teneriffa und La Gomera sowie eine südwestliche Gruppe auf La Palma und El Hierro. Aus dieser markanten Differenzierung resultiert die Beschreibung eines neuen Taxon von El Hierro und La Palma *Regulus regulus ellenthalerae* n. ssp.

Für die Blaumeisen wird eine Trennung der nördlichen *P. caeruleus* (Europe) von der südlichen *P. teneriffae* – Gruppe (Nordafrika, Kanarische Inseln) empfohlen. Dabei sollten *degener* und *ultramarinus* als synonym (*P. teneriffae ultramarinus*) betrachtet werden und ein neues Taxon wurde auf Gran Canaria festgestellt und beschrieben (*P. t. hedwigii* nov. ssp.).

Die subspezifische Diagnostizierbarkeit für Samtkopf- (*Sylvia melanocephala*) und Mönchsgrasmücken (*S. atricapilla*) von den Atlantischen Inseln wurde verworfen. Morphologische Unterschiede sind bei beiden Arten eher in Zugverhalten und Ökologie begründet und spiegeln nicht die Phylogenie wieder. Vorläufige Daten einer kleinen Stichprobe suggerieren ebenfalls eine geringe genetische Differenzierung bei der Brillengrasmücke (*S. conspicillata*).

1 Introduction

The field of phylogeography (Avise et al. 1987) seeks to describe biogeographic patterns of genetic structure and to infer the history and processes underlying this structure (Avise 2000, Knowles & Maddison 2002). A detailed knowledge of the evolution and geographic variation of taxa helps to identify lineages of independent evolutionary history, which in turn may contribute to our efforts to classify and conserve biodiversity. A first step to improve our understanding of how species evolve and diverge is to study the genetic differentiation and geographic association of genealogical lineages.

Islands have become synonymous with the study of evolution since the famous association between Darwin's theory of evolution by natural selection and the finches of the Galapagos islands. Island systems are attractive environments for studying evolution for a number of reasons: (1) they present discrete geographical entities within defined oceanic boundaries; (2) gene flow between individual islands is reduced by oceanic barriers; (3) their often small size has made the cataloguing of fauna and flora easier than continental systems; (4) despite their small geographical size they can host a diversity of habitats and; (5) they are often geologically dynamic with historical and contemporary volcanic and erosional activity (Emerson 2002). The rich species diversity of island biota is the result of a number of factors: (1) the diversification of a founding population into an array of species differentially adapted to diverse environmental niches (adaptive radiation); (2) multiple successful colonizations to an island from neighbouring islands or a continental land mass; (3) the diversification of a founding population into a number of species caused by vicariance events such as lava flows and erosional events and; (4) increased speciation through bottleneck and founder flush events (Templeton 1980, Carson & Templeton 1984, Emerson 2002)

The main aim of the present study was to investigate the degree of differentiation and phylogeographic relationships of selected passerine bird species on the Atlantic islands, particularly the Canary Islands.

1.1 Geography and geology of Macaronesia

Macaronesia is a modern collective name for several groups of islands in the North Atlantic Ocean near Europe and North Africa belonging to the three countries: Portugal, Spain, and Cape Verde (Figure 1). The name comes from the Greek for "blessed islands", a term used by Ancient Greek geographers for islands to the west of the Straits of Gibraltar (Clarke 2006). Macaronesia consists of five archipelagos: (1) the Azores (Portugal), (2) Madeira (Portugal), (3) The Canary Islands (Spain), (4) Cape Verde (Cape Verde) and (5) the Salvage Islands (Portugal). The oceanic islands of Macaronesia are of volcanic origin as a product of several geologic hotspots. The climate ranges from subtropical to tropical.

The Portuguese archipelagos of the Azores and Madeira have a generally cooler climate and higher rainfall than the Canary and Cape Verde Islands.

Figure 1 Macaronesia and included archipelagos
From Clarke (2006).

The islands reveal a unique biogeography and are home to several distinct plant and animal communities with a high proportion of endemism. Since none of the volcanic Macaronesian islands were connected to the European or African continents (Abdel-Monem et al. 1971, Kunkel 1976, Schmincke 2000), the native plants and animals must have reached the islands via long-distance dispersal. Thus, the Canary Islands are by all means comparable to the Galapagos Islands, which since the initial investigations of Charles Darwin have become famous examples for evolutionary and speciation processes on oceanic island biotas (Grant 1986, 1998, 2001).

Laurel-leaved forests, called laurisilva, once covered most of the Azores, Madeira and parts of the Canary Islands between altitudes of 400-1 200 m a.s.l. These forests resemble the ancient forests that covered the Mediterranean basin and northwestern Africa before cooling and drying during the ice ages. Trees of the genera *Apollonias* (Lauraceae), *Clethra* (Clethraceae), *Dracaena* (Ruscaceae), *Ocotea* (Lauraceae), *Persea* (Lauraceae) and *Picconia* (Oleaceae), which are found in the laurisilva, are also known from fossils around the Mediterranean before the ice ages. Harvesting of the forests for timber and firewood, clearing vegetation for grazing and agriculture and the introduction of exotic plants and animals by humans has displaced much of the native vegetation. The laurisilva has been reduced to small pockets. As a result, many of the endemic biota of the islands are seriously endangered or even extinct (Fernandez 2001).

This study mainly focusses on the archipelago of the Canary Islands and, if applicable, also Madeira and the Azores.

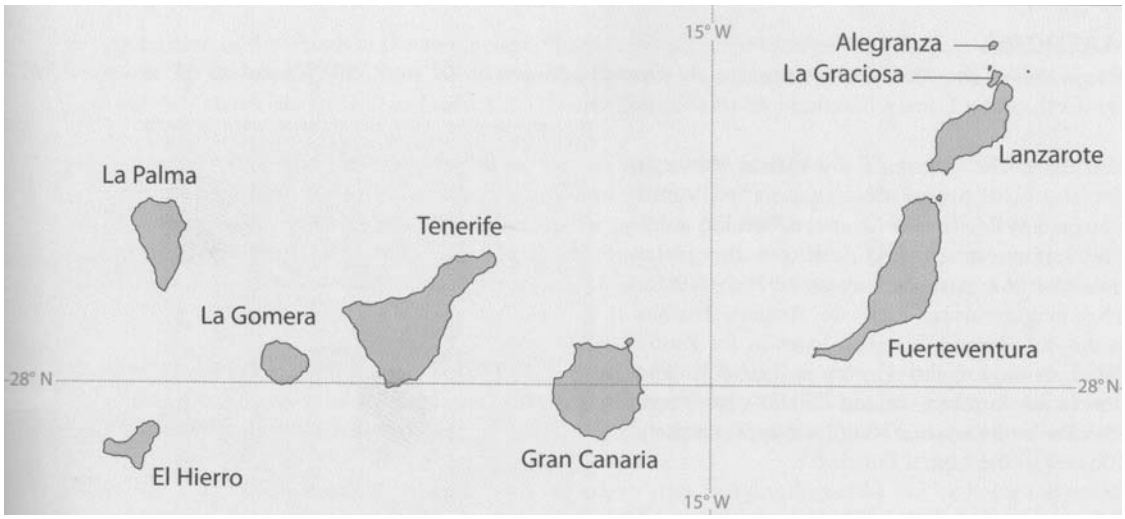

Figure 2 Canary Islands (Spain)
From Clarke (2006).

1.1.1 Canary Islands

The Canary Islands are located in the eastern Atlantic between 13°23' and 18°08' W and 27°37' and 29°24' N (Figure 2). The archipelago consists of seven main islands (Table 1): Fuerteventura, Lanzarote, Gran Canaria, Tenerife, La Gomera, El Hierro and La Palma as well as some small islets, covering an area of 7 490 km². The largest island is Tenerife (2 034 km²) while La Graciosa covers only 27 km². The highest peaks are found on Tenerife (Mt. Teide 3 718 m a.s.l.) and La Palma (2 423 m a.s.l.) whereas the eastern islands do not exceed 1 000 m a.s.l. (Martin & Lorenzo 2001).

The islands are of volcanic origin, which together with repeated periods of erosion and construction, have resulted in a high diversity of landscapes and habitats. Particularly the drier easternmost islands of Fuerteventura and Lanzarote contrast strongly to the more humid central and western islands with extensive laurisilva.

Prevailing thought on the origin of the Canary Islands holds that the islands were formed on a submarine geological hotspot, which moved from east to west and volcanic eruptions and tectonic updrifts gave rise to the islands. The islands were not created by one geological event, but rather by a series of consecutive events along the track of the hotspot. Consequently, the oldest islands are Fuerteventura (20 my) and Lanzarote (16 my) followed by Gran Canaria (15 my), Tenerife (12 my), La Gomera (10 my), La Palma (2 my) and finally El Hierro (1 my). Ongoing geologic events continued forming the islands into the 20th century. The formation of the islands was sometimes very complex, for example Tenerife originates from three small precursor islands – Roque del Conde, Teno and Anaga massifs – which were later connected by a giant eruption and consequent landslides formed the island as it is known today (Juan et al. 2000).

The subtropic climate is influenced by superior factors like the Azorean anticyclon and the cold Canarian current as well as more local factors like altitude and orientation. On the central and western islands the combination of northeastern and southwestern trade winds creates a typical zone of clouds between 800 and 1 500 m a.s.l. which is dominated by laurel cloud forests. The southern parts of the islands show a warmer and drier climate. Particularly the eastern islands are very dry and desert like (annual rainfall on Fuerteventura and Lanazrote is 111 and 162 mm, respectively, reminiscent of desert areas in northwest Africa and compared to 731 mm on La Palma; Martin & Lorenzo 2001).

Table 1 Characterisation of Macaronesian archipelagos of the Canary Islands, Madeira and the Azores [a)]

Island	Area [km²]	Maximum altitude [m a.s.l.]	Maximum estimated geological Age [my]	Distance from continent [km]
Canary Islands (Spain)				
Fuerteventura	1725	807	21	94
Lanzarote	796	671	15.5	131
Gran Canaria	1532	1 950	14	188
Tenerife	2058	3 718	11.6	263
La Gomera	378	1 482	12	313
La Palma	729	2 426	2	375
El Hierro	269	1 501	1	350
Madeira (Portugal)				
Madeira	742	1 862	5.2	640
Porto Santo	43	517	14	625
Desertas	14	478	5.2	630
Salvage Islands	3	154	11	270
Azores (Portugal)				
Santa Maria	97	587	6	1 343
São Miguel	750	1 103	4	1 358
Terceira	400	1 021	3.5	1 552
Graciosa	62	398	2.5	1 625
São Jorge	246	1 067	0.6	1 614
Pico	436	2 351	0.3	1 640
Faial	173	1 043	0.7	1 688
Corvo	17	718	0.7	1 890
Flores	143	913	0.7	1 898

a) According to Hughes & Malmqvist (2005)

The distance of the islands from the adjacent African mainland varies between 110 (Fuerteventura) and 380 km (La Palma), while inter-island distances between neighbouring

islands range from 11 (Fuerteventura – Lanzarote) to 81 km (Fuerteventura – Gran Canaria). The islands apparently have never been connected to the continent (water depth between Fuerteventura and Africa 1 500 m) or to each other (water depth up to 3 000 m), maybe except Fuerteventura and Lanzarote, which are only separated by depths of 200 m.

1.1.2 Madeira

The Madeiran archipelago is located about 400 km to the northeast of the Canary Islands and 900 km to the southeast of the Azores at 30°01' and 33°08' N and 15°51' and 17°16' W. It consists of the main island Madeira and the smaller islands Porto Santo and Ilhas Desertas. Generally, also the Salvage Islands – although much closer to the Canary Islands – are officially part of the Madeiran archipelago (Clarke 2006). The total area of the archipelago is less than 1 000 km² and Madeira alone covers an area of 740 km² (Table 1).

Like the Canary Islands, Madeira and Porto Santo are of volcanic origin. The islands were built up during consecutive periods of volcanic activity during 2.5 – 65 my ago and latest eruptions are dated to the early Pleistocene (< 2 my ago). The nearby Ilhas Desertas were connected to the island of Madeira in the past, while Porto Santo has always been separated (Röpke & Senne 2000).

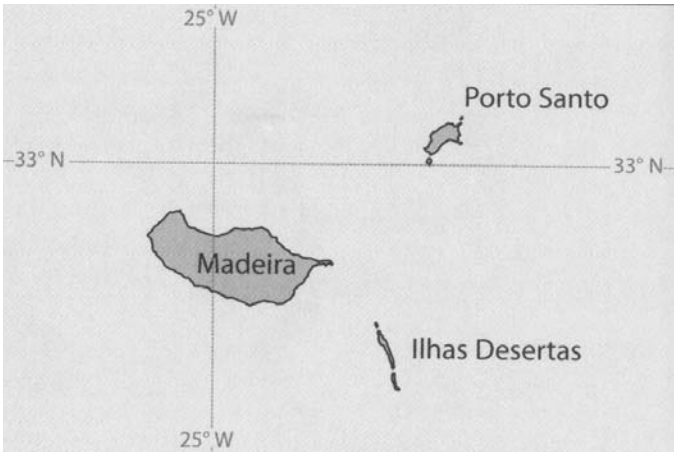

Figure 3 Madeiran archipelago
From Clarke (2006).

The subtropical climate is similar to the Canary Islands and is caused by the same factors, i.e. the cold Canarian current and the trade winds. The north side of Madeira and the high altitudes are more humid than the south with average annual rainfalls of 2 246 mm and 513 mm, respectively (Röpke & Senne 2000).

1.1.3 The Azores

The Azores are situated in the mid Atlantic between 36°55' and 39°43 N and 25°01' and 31°07' W, comprising nine islands in three distinct groups at the western edge of the Westpalearctic. The eastern group contains the islands of Santa Maria and São Miguel, the central group contains Terceira, Graciosa, São Jorge, Pico and Faial, and the western group contains Corvo and Flores. The total area of the Azores covers 2 250 km² stretching over 480 km from east to west. Santa Maria is situated 1 343 km to the west of the nearest continental land in Portugal (Hughes & Malmqvist 2005). The distance between

neighbouring islands varies from 7 – 88 km, while island groups are 145 to 190 km apart (Clarke 2006).

Like in the Canary Islands, the geological age of the Azores increases along a gradient from the west to the east, but the Azorean islands are generally much younger than the Canary Islands ranging from 0.3 (Pico) to 6 my (Santa Maria).

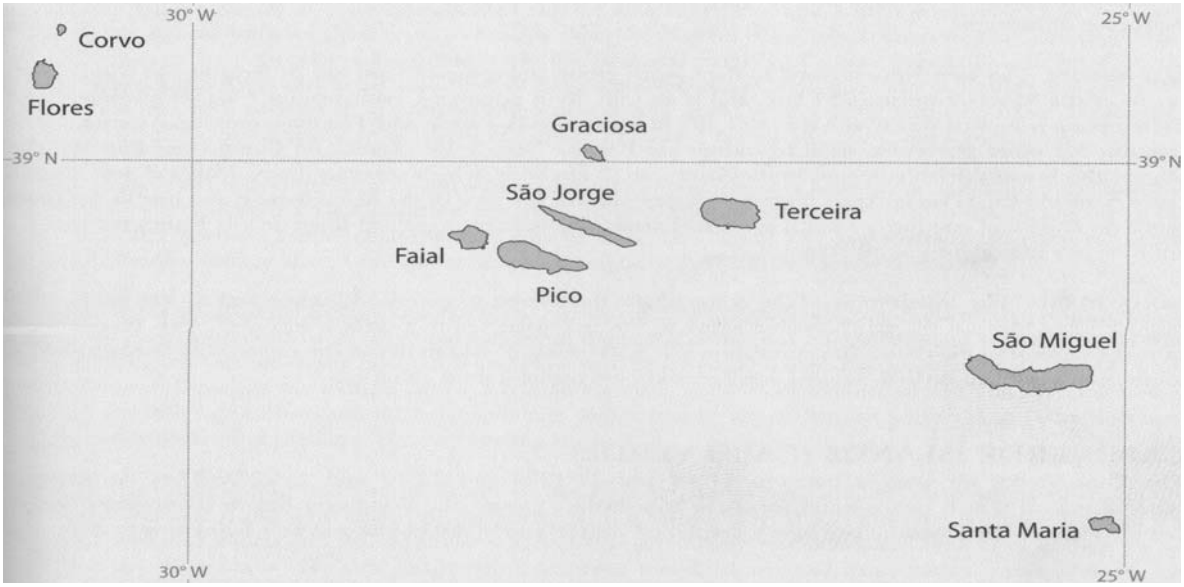

Figure 4 **The Azores**
From Clarke (2006).

The archipelago's location in the north Atlantic brings it in constant contact with both the high pressure areas common to the North Atlantic and the constantly moving polar and tropical air masses. As the European continent is over 1 500 km away oceanic conditions are the controlling factors of the regional climate[i]. Consequently, the Azores have a temperate, maritime climate characterised by agreeable temperatures with small average annual variance. A high level of humidity (77 % annual average) and rainfall – which is both regular and well distributed throughout the year although with greater abundance during the winter months – are the dominant weather patterns in the region. Light cloud cover is common throughout the year. The annual median air temperature is approximately 17 ºC, varying between 13 ºC and 14 ºC during the colder months (January/February) and 22 – 23 ºC during the warmer months (July/August). The ocean temperatures oscillate between 14 – 15 ºC during the coldest month (February) and between 22 – 23 ºC in August largely due to the warming effects of the Gulf Stream that passes this area.

[i] Data on climate according to http://www.destinazores.com/climate.php

1.2 Phylogeography of Macaronesian faunal elements

Until very recently, the Macaronesian islands generally have been ignored by evolutionary studies although a large proportion of endemic species has been known long before (Meade-Waldo 1893, Volsoe 1955, Bannermann 1963, Bannermann & Bannermann 1965, 1966, 1968). However, recent studies have shown that, regarding evolutionary processes particularly the Canary Islands do not stand behind, for example, the Galapagos Islands. There are several reasons why oceanic islands and thus also the Canary Islands are prime locations for evolutionary studies: (1) the geographic isolation is appreciated as a main factor in speciation processes, (2) the diversity of habitats and ecological niches leaves possibilities for island radiations and (3) the knowledge about the geological history of the islands presents a chronological time frame for evolutionary events (Juan et al. 2000).

First results of molecular phylogenetic and phylogeographic studies on the Canary Islands were published in the early 1990s. The number of phylogeographic studies has been increasing ever since. Intitial studies mainly focused on flightless arthropods and reptiles, which both show high species diversities on the Canary Islands. These studies mainly investigated (1) colonization pathways and the stepping-stone model, (2) phylogeographic patterns on individual islands and the influence of volcanic incidences and (3) radiations, habitat shifts and adaptations (Juan et al. 2000).

Many molecular studies revealed a stepwise colonization of the Canary Islands progressing from the older easternmost islands towards the younger western islands. Good examples for the stepping-stone model were found in the lizard genus *Gallotia* (Thorpe et al. 1993a, b, Thorpe et al. 1994a, Gonzalez et al. 1996), the tenebrinoid beetle genera *Pimelia* (Juan et al. 1995, Juan et al. 1996b) and *Hegeter* (Juan et al. 1996a). The endemic weevil genus *Brachyderes* is associated with native pine forests and the colonization of the Canary Islands also complies with the stepping-stone model (Emerson et al. 2000). A similar pattern is displayed by lepidopterans of the genus *Gonepteryx* (Brunton & Hurst 1998) and *Drosophila* flies (Khadem et al. 1998). However, this simple pattern is often clouded by re-colonizations, multiple colonizations, island-specific differentiations, adaptations and extinctions as in the beetle genus *Calathus* (Emerson et al. 1999), the skink genus *Chalcides* (Brown & Pestano 1998) and geckos of the genus *Tarentola* (Nogales et al. 1998, Gübitz et al. 2000).

After the initial colonization of an island the specific differentiation is mainly affected by vicariance caused by lava flows and local extinctions, followed by re-colonizations. The islands of Lanzarote and Fuerteventura were formed by two distinct periods of prominent volcanic activity at 21-12 my BP and six my BP, respectively. Those volcanic activities resulted in a gradient of older (southwest) to younger (northeast) surface strata. The geological age gradient is reflected by the mitochondrial genealogy of the endemic beetle

Hegeter politus, where the ancestral population in the southwest followed the ceasing volcanic activity (Juan et al. 1998). The specific geologic history of Tenerife, with smaller precursor islands that were later joined, is crucial for the understanding of island-specific differentiation. Many organisms show vicariant sister taxa in the Teno and Anaga massifs of Tenerife. The gecko *Tarentola delalandii* shows three distinct lineages which can be correlated to the Teno, Roque del Condo and Anaga regions from where central parts of the island were colonized later (Nogales et al. 1998, Gübitz et al. 2000). A similar pattern was found for the skink *Chalcides viridanus*, with ancestral populations in the Anaga and Teno regions (Brown et al. 2000). Both *Tarentola* and *Chalcides* show distinct morphological and genetic differentiations on Gran Canaria which can be correlated to vicariance during the last volcanic period 2.8 my BP. There are several hints for a mass extinction on Gran Canaria 3.4-4.5 my ago (Nogales et al. 1998, Brown et al. 2000, Gübitz et al. 2000).

Oceanic islands in general and the Canary Islands in particular show a large diversity of habitats, which in combination with the isolated location, offer prime preconditions for species radiations. Many such radiations have been documented for plants, e.g. *Argyranthemum*, *Sonchus* and *Echium*. The Canary Islands display a large proportion of troglomorphic spiders in the genus *Dysdera* which are all derived from epigaeic ancestors following 3-4 independent colonization events (Arnedo & Ribera 1997). The beetle genera *Calathus* and *Nesotes* are presented on the Canary Islands by 24 and 19 species, respectively, and genetic data point towards a recent diversification through marginal niche specialization in micro habitats by generalist ancestors (Emerson et al. 1999, Rees et al. 2001a, b, c, Emerson 2002). Generally, stochastic factors are an essential parameter for determination of species composition on different islands. After successful colonization of an island, subsequent evolution depends on occurrence or absence of other species, competition, volcanic incidents and adaption potential (Emerson et al. 1999).

It was assumed until very recently that no native mammals occurred on the Atlantic Islands, although fossil bone fragments had been found in 1940. After 1983, two species of *Crocidura* had been described. Both species are not closely related to each other. Both have relatives in the Mediterranean basin and North Africa, however, and have colonized the Canary Islands independently (Hutterer 1992).

There have been several studies investigating the differentiation of Macaronesian bird species compared to related taxa on the continent, including Canarian Chiffchaff *Phylloscopus canaria* (Helbig et al. 1996), Houbara Bustard *Clamydotis undulata* (Gaucher et al. 1996, Idaghdour et al. 2004), Canary Island Stonechat *Saxicola dacotiae* (Wittmann et al. 1995), Berthelot's Pipit *Anthus berthelothi* (Alström & Mild 1993, Arctander et al. 1996). The only bird species investigated with regard to its molecular phylogeography and intraspecific differentiation on the Atlantic islands was the chaffinch

(*Fringilla coelebs*), which is represented by several morphologically distinct subspecies – *F. c. moreletti* (Azores), *F. c. maderensis* (Madeira), *F. c. canariensis* (Gran Canaria, Tenerife, La Gomera) und *F. c. palmae* (La Palma, El Hierro). These island populations form a monophyletic group and the colonization of Macaronesia originated on the European mainland from where the Azores, Madeira and finally the Canary Islands were colonized (Marshall & Baker 1999). Within the Canary Islands birds initially arrived on the western islands of La Palma and El Hierro, from where the central islands La Gomera and Tenerife were subsequently colonized. The island of Gran Canaria was invaded last and the colonization of the Canary Islands does not comply with the stepping-stone model from older to younger islands. The colonization process was a rapid sequence of events that took place rather recently. There is also evidence for back-colonizations, e.g. on Madeira (Marshall & Baker 1999).

Of course, phylogeographic investigations on the Canary Islands are not restricted to faunal elements and a number of studies elucidating colonization patterns, radiations and island specific adaptations in numerous plant species are available as well (Bohle et al. 1996, Francisco-Ortega et al. 1996, Hess et al. 2000, Gomez et al. 2003, Allan et al. 2004, Guzman & Vargas 2005, Trusty et al. 2005, Navascues et al. 2006).

1.3 Avifauna of Macaronesia

As of 2005 totals of 429, 293 and 279 bird species have been recorded on the Canary Islands, Madeira and the Azores, respectively (Martin & Lorenzo 2001, Clarke 2006). Most of these species occur as more or less regular migrants or rare vagrants, while the number of breeding species is much lower (98, 37 and 38, respectively). The Canary Islands host six endemic species, a further 29 endemic subspecies and four endemic species are shared with other Atlantic islands (Table 2). Madeira is home to three endemic species, while a further five species are Macaronesian endemics. Only one species is totally confined to the Azores, where three more Macaronesian endemics occur (Table 2).

The number of breeding songbirds (Order Passeriformes) is rather low on all archipelagos (Canary Islands 33 spp., Madeira 14 spp., Azores 13 spp.) and few species (n = 15) are shared between at least two archipelagos, while seven occur on all three (Clarke 2006). The selection of species for the present study was based on (1) expected high genetic differentiation for species with morphologically distinct subspecies, (2) similtaneous distribution on several islands, archipelagos and the continental mainland to investigate degrees of inter-island differentiation and possible colonization pathways and (3) chances of collecting adequate numbers of samples for meaningful analysis of results. With these aspects in mind, the following taxa have been selected for this initial study:

- European robin (*Erithacus rubecula*)
- Island canary (*Serinus canaria*)

- Goldcrests (*Regulus* spp.)
- Blue tit (*Parus teneriffae* – group)
- Representatives of the genus *Sylvia*, namely Sardinian warbler (*S. melanocephala*), blackcap (*S. atricapilla*) and spectacled warbler (*S. conspicillata*)

Table 2 Endemic bird species and subspecies on the Macaronesian archipelagos
After Clarke (2006) and Martin & Lorenzo (2001).

Archipelago	Endemic species	Endemic subspecies	Macaronesian endemics
Canary Islands	Bolle's pigeon (*Columba bollii*) Laurel pigeon (*Columba junoniae*) Canary island stonechat (*Saxicola dacotiae*) Canary island chiffchaff (*Phylloscopus canariensis*) Tenerife kinglet (*Regulus teneriffae*) Blue chaffinch (*Fringilla teydea*)	Common buzzard (*B. b. insularum*) Common kestrel (*F. t. dacotiae*, *F. t. canariensis*) Houbara bustard (*C. u. fuerteventurae*) Stone curlew (*B. o. insularum*, *B. o. distinctus*) Barn owl (*T. a. gracilirostris*) Long-eared owl (*A. o. canariensis*) Great spotted woodpecker (*D. m. canariensis*, *D. m. thanneri*) Lesser short-toed lark (*C. r. polatzeki*) Grey wagtail (*M. c. canariensis*) Robin (*E. r. superbus*) Blackbird (*T. m. cabrerae*) Spectacled warbler (*S. c. gularis*) Sardinian warbler (*S. m. leucogastra*) Blue tit (*P. t. teneriffae*, *P. t. degener*, *P. t. palmensis*, *P. t. ombriosus*) Southern grey shrike (*L. m. koenigi*) Chaffinch (*F. c. canariensis*, *F. c. palmae*, *F. c. ombrosus*) Linnet (*C. c. meadewaldoi*, *C. c. harteti*)	Madeiran storm-petrel (*Oceanodroma castro*) Macaronesian shearwater (*Puffinus baroli*) Berthelot's pipit (*Anthus berthelotii*) Island canary (*Serinus canaria*)
Madeira / Salvages	Zino's pPetrel (*Pterodoma madeira*) Trocaz pigeon (*Columba trocaz*) Madeira firecrest (*Regulus madeirensis*)	Barn owl (*T. a. schmitzi*) Grey wagtail (*M. c. schmitzi*) Chaffinch (*F. c. madeirensis*) Linnet (*C. c. guentheri*)	Fea's petrel (*Pterodroma feae*) Madeiran storm-petrel (*Oceanodroma castro*) Macaronesian shearwater (*Puffinus baroli*) Berthelot's pipit (*Anthus berthelotii*) Island canary (*Serinus canaria*)
The Azores	Azores bullfinch (*Pyrrhula murina*)	Grey wagtail (*M. c. patriciae*) Blackbird (*T. m. azoricus*) Blackcap (*S. a. gularis*) Goldcrest (*R. r. sanctaemariae* *R. r. azoricus*, *R. r. inermis*,) Common starling (*S. v. granti*) Chaffinch (*F. c. moreletti*)	Madeiran storm-petrel (*Oceanodroma castro*) Macaronesian shearwater (*Puffinus baroli*) Island canary (*Serinus canaria*)

A short overview on the taxonomy and distribution of these taxa is presented in the following chapters. Further details are provided in the respective contributions (chapters 3.1 to 3.5).

1.3.1 European robin (*Erithacus rubecula*)

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 (Figure 5). 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* (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' (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).

The species is generally widespread and common on all three archipelagos, particularly on the Azores, where all islands except Corvo and Flores are inhabited (Clarke 2006). On Madeira the Robin only breeds on the main island of Madeira but it has also been recorded on Porto Santo and the Salvages (Clarke 2006). It is also a common breeding species on all Canary Islands except Fuerteventura and Lanzarote. The preferred habitat on all archipelagos are native forests and shrublands (laurisilva, tree heath) but it is scarce in the high altitude pure pine forests of, for example, Tenerife (Martin & Lorenzo 2001).

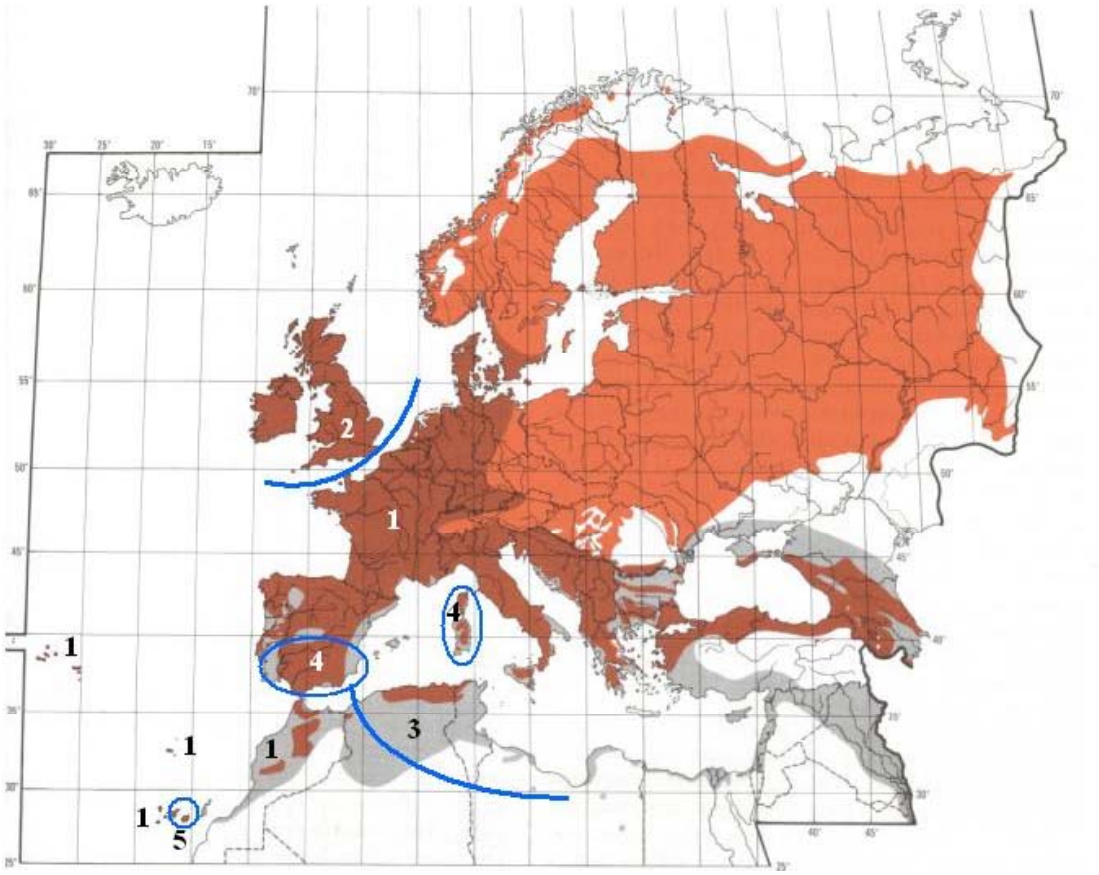

Figure 5 **Distribution of the European robin (*Erithacus rubecula*) in the Westpalearctic**
From Cramp (1988). Pale red = breeding, grey = wintering, dark red = resident throughout the year. (1) = *E. r. rubecula*, (2) = *E. r. melophilus*, (3) = *E. r. witherby*, (4) = *E. r. sardus*, (5) = *E. r. superbus*.

1.3.2 Island Canary (*Serinus canaria*)

The distribution of the monotypic Island (Atlantic) canary (*Serinus canaria*) is restricted to Macaronesia, where it is a fairly common resident on all archipelagos except Cape Verde (Figure 6). On the Azores all islands are occupied and it is also a well distributed breeding species on Madeira, Porto Santo and the Desertas. Except for the eastern islands of Fuerteventura and Lanzarote, the Island Canary is very abundant and common on all Canary Islands. The populations on Fuerteventura and Lanzarote are very small and localised (Martin & Lorenzo 2001, Clarke 2006).

The Island Canary occurs in all kinds of habitats from sea level to high mountains including laural and pine forests, tree heath, gardens, parks, orchards (Martin & Lorenzo 2001, Clarke 2006).

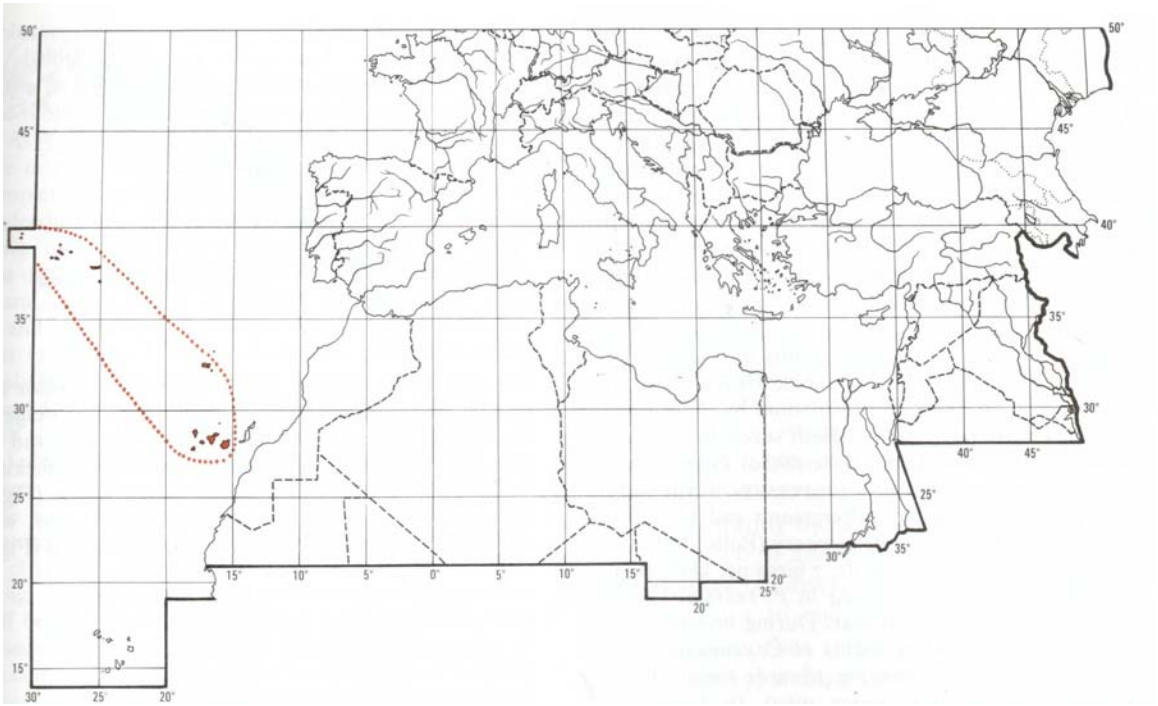

Figure 6 Distribution of the Island Canary (*Serinus canaria*) in the Westpalearctic
From Cramp & Perrins (1994).

1.3.3 Goldcrests (*Regulus* spp.)

The genus *Regulus*, formerly included into the Sylviidae, is now generally positioned in a family of its own including up to six species with a mainly Holarctic distribution (Clements 2000). The Golden-crowned Kinglet (*R. satrapa*) and Ruby-crowned Kinglet (*R. calendula*) occur in North-America, while the Flamecrest (*R. goodfellowi*) is restricted to montane forests of central Taiwan. Two species occur in the Westpalearctic, the goldcrest (*R. regulus*) is widely distributed from the Canary Islands in the west to central Siberia in the east (Figure 7), including up to 15 more or less distinct subspecies (Thaler 1990, Clements 2000). Birds from the Canary Islands (*R.* [*r.*] *teneriffae*) are often treated as a separate species, while birds from the Azores – and also those from Taiwan – are usually included in *R. regulus*. The distribution of the firecrest (*R. ignicapillus*) is confined to the western Palearctic region (Figure 8) but both species often occur sympatrically. The birds from Madeira are usually included in *R. ignicapillus* (Thaler 1990).

Consequently, each archipelago is inhabited by a different *Regulus* taxon. On the Canary Islands the Tenerife goldcrest (*R.* [*r.*] *teneriffae*) breeds on Tenerife, La Gomera, El Hierro and La Palma, where it inhabits laurel as well as pine forests, but highest densities are found in tree heath with *Erica arborea* and *E. scoparia* (Martin & Lorenzo 2001, Clarke 2006). On the Azores all islands except Graciosa and Corvo are inhabited by the goldcrest (*R. regulus*), which is presented by three subspecies, namely *R. r. sanctamariae* (Santa Maria), *R. r. inermis* (Flores, Faial, Pico, São Jorge, Terceira) and *R. r. azoricus*

(São Miguel). Preferred habitats include wooded areas and areas with tall shrubs including junipers (Clarke 2006).

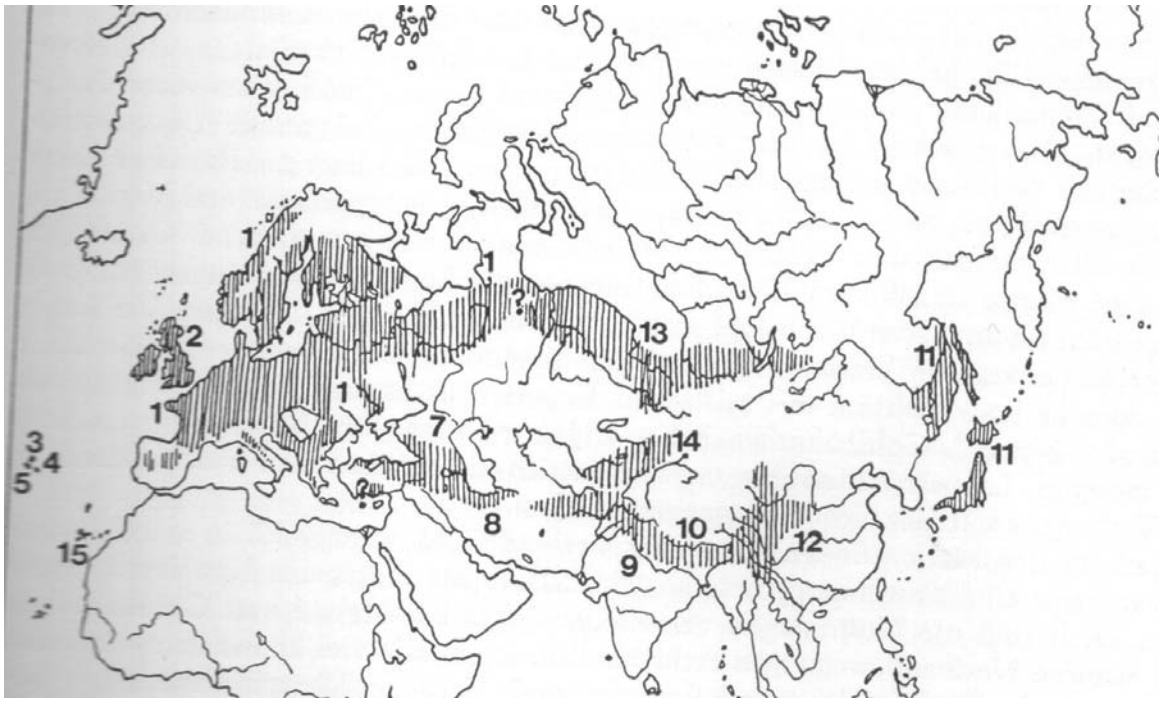

Figure 7 **Distribution of the goldcrest (*Regulus regulus* ssp.) in the Palearctic**
From Thaler (1990). Numbers indicate distribution of (sub)species: 1 = *regulus*, 2 = *anglorum*, 3 = *azoricus*, 4 = *sanctamariae*, 5 = *inermis*, 6 = *interni*, 7 = *buturlini*, 8 = *hyrcanus*, 9 = *himalayaensis*, 10 = *sikkimensis*, 11 = *japonicus*, 12 = *yunnaensis*, 13 = coatsi, 14 = *tristis*, 15 = *teneriffae*.

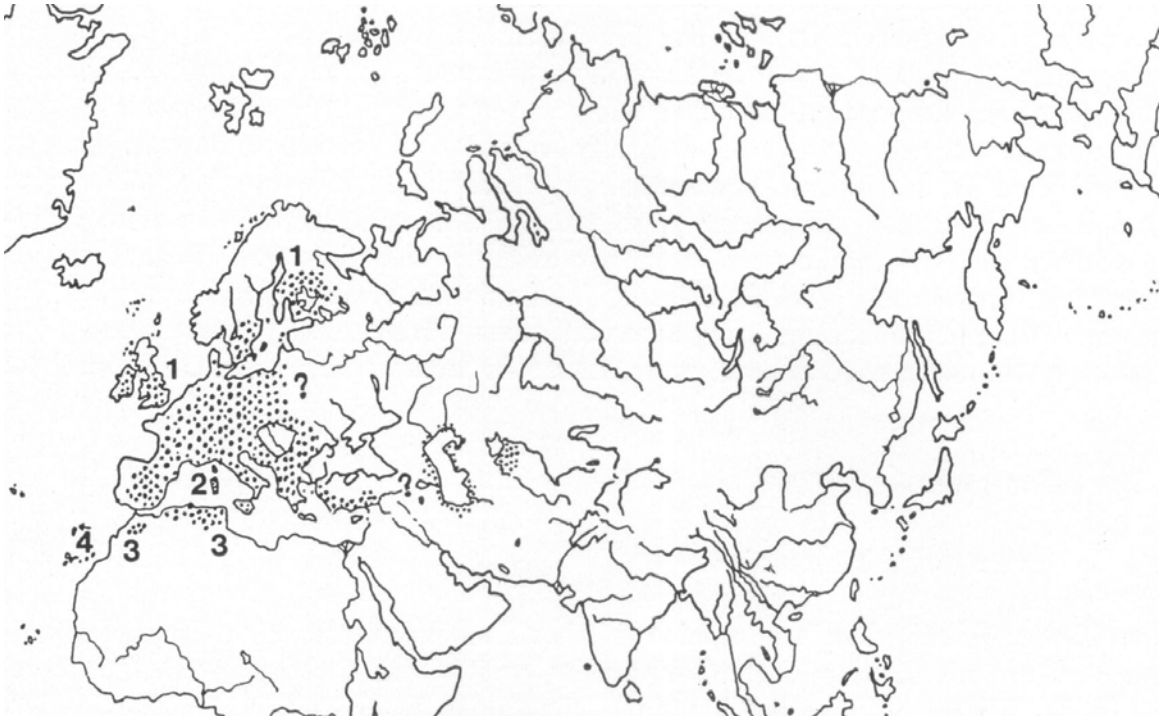

Figure 8 **Distribution of the firecrest (*Regulus ignicapillus*) in the Westpalearctic**
From Thaler (1990). Numbers indicate distribution of (sub)species: 1 = *ignicapillus*, 2 = *balearicus*, 3 = *laeneni*, 4 = *madeirensis*.

The Madeiran firecrest (*R.* [*i.*] *madeirensis*) is confined to the main island of Madeira where all wooded habitats are occupied with a preference of tree heaths at higher altitudes (Clarke 2006).

1.3.4 Blue tit (*Parus teneriffae* – group)

The polytypic Blue tit (*P. caeruleus*) is distributed over large parts of Europe from the Canary Islands to the Ural Mountains including North Africa and Asia Minor (Cramp & Perrins 1993, Glutz von Blotzheim & Bauer 1993). Traditionally, at least 15 subspecies are recognised with nominate *caeruleus* in northern, central and eastern Europe south to northern Spain, Italy, Greece and Asia Minor, *P. c. ogliastrae* in southern Iberia, Corsica and Sardinia, *P. c. ultramarinus* in northwestern Africa and the four Canary Island taxa *ombriosus* (El Hierro), *palmensis* (La Palma), *teneriffae* (La Gomera, Tenerife, Gran Canaria) and *degener* (Fuerteventura, Lanzarote) to name just a few (Dickinson 2003). Recent molecular studies gave evidence for splitting the northern *caeruleus*–group from the southern *teneriffae*–group, including *ultramarinus* and the Canary Island taxa (Salzburger et al. 2002a, Kvist et al. 2004) which is also followed here. The same authors have furthermore suggested conspecificity of the closely related Azure tit (*P. cyanus*) and Yellow-breasted tit (*P. flavipectus*). The three species *caeruleus/teneriffae*, *cyanus* and *flavipectus* are subsumed in the (sub)genus *Cyanistes* (Clements 2000, Gill et al. 2005).

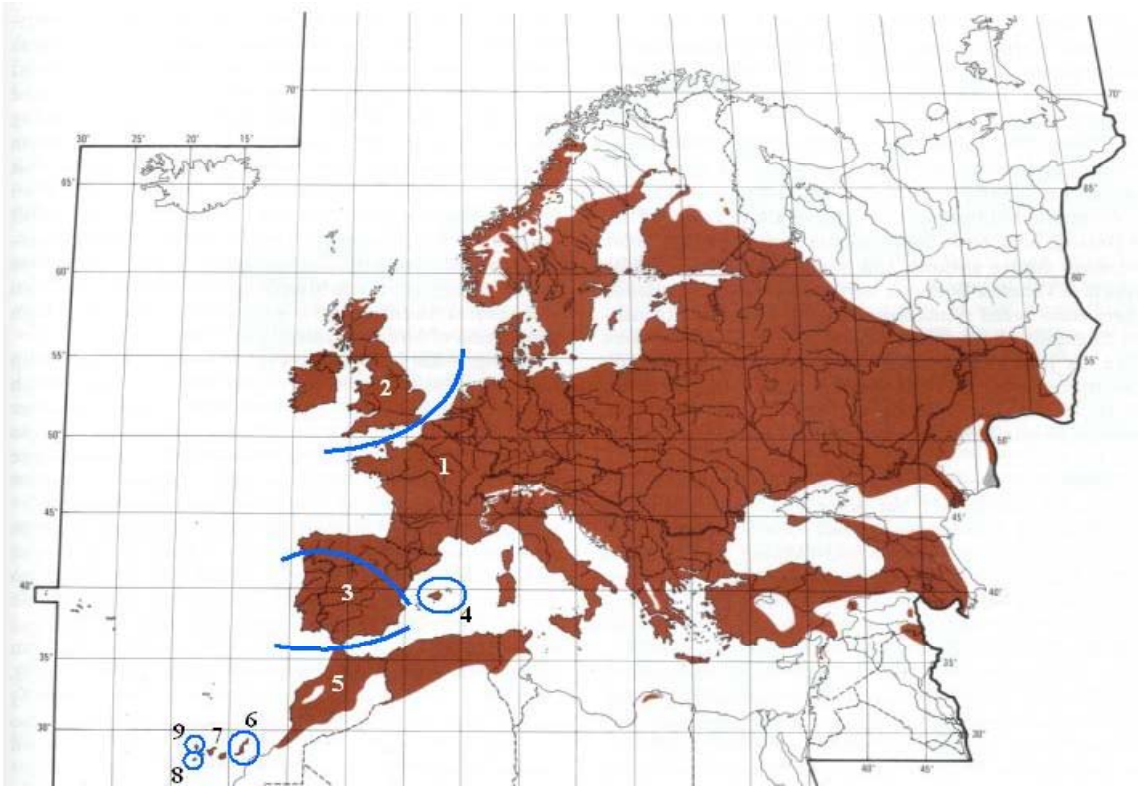

Figure 9 **Distribution of the blue tit (Parus caeruleus) in the Westpalearctic**
From Cramp & Perrins (1993). (1) = *P. c. caeruleus*, (2) = *P. c. obscurus*, (3) = *P. c. oglistrae*, (4) = *P. c. balearicus*, (5) = *P. teneriffae ultramarinus*, (6) = *P. t. degener*, (7) = *P. t. teneriffae*, (8) = *P. t. ombriosus*, (9) = *P. t. palmensis*.

Within Macaronesia only the Canary Islands are inhabited by the blue tit which occurs from sea level to high altitudes in laurel forests, pine forests, tree heath, parks and gardens. The abundance is higher on the well vegetated central and western islands but less so on the desert like eastern islands (Martin & Lorenzo 2001, Clarke 2006).

1.3.5 Warblers of the genus *Sylvia*

The Sardinian warbler (*S. melanocephala*) is distributed around the Mediterranean Sea including coastal areas of southern Europe, the Middle East, North Africa and the Canary Islands (Shirihai et al. 2001). Four subspecies have been described: *S. m. leucogastra* (Canary Islands), *S. m. melanocephala* (S Europe, Mediterranean islands, Turkey and North Africa), *S. m. norrisae* (formerly Egypt, extinct) and *S. m. momus* (Syria, Israel, Jordan and Sinai Peninsula; Clements 2000). It is found on all Canary Islands in areas with sufficient vegetation, but it avoids towns, villages, desert scrub and high mountains (Clarke 2006).

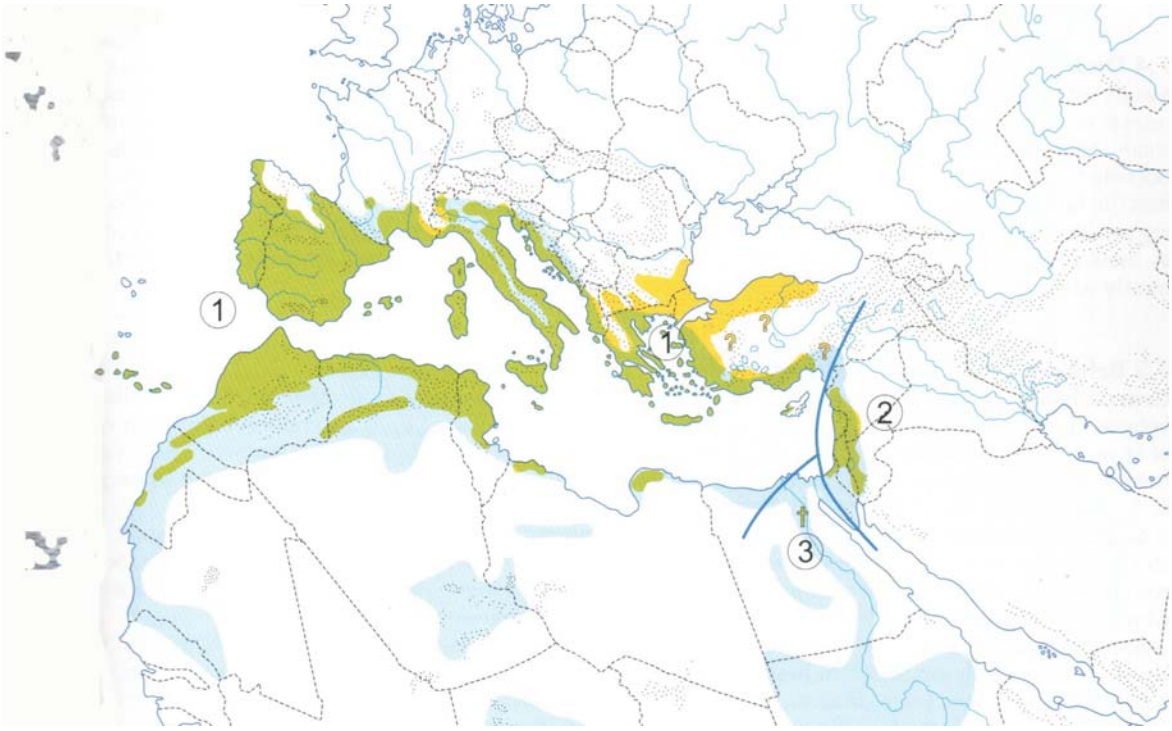

Figure 10 Distribution of the Sardinian warbler (*Sylvia melanocephala*) in the Westpalearctic
From Shirihai et al. (2001). (1) *S. m. melanocephala*, (2) *S. m. norrisae*, (3) *S. m. momus*.

The blackcap (*S. atricapilla*) shows a Palearctic distribution from the Atlantic Islands in the west to the Caucasus in the east (Figure 11) with five recognised subspecies: *S. a. gularis* (Cape Verde Islands, Azores), *S. a. heineken* (Spain, Portugal, North Africa, Madeira, Canary Islands), *S. a. atricapilla* (Europe to Siberia), *S. a. pauluccii* (Corsica, Sardinia, Balearics, Italy, Tunisia, Sicilly) and *S. a. dammholzi* (Caucasus, Transcaucasia,

Iran; Clements 2000, Shirihai et al. 2001). The blackcap is a common resident on all archipelagos including the Azores (all islands), Madeira with the Salvages and the Canary Islands (central and western islands). The status on Fuerteventura and Lanzarote is unclear and it is here, and on Tenerife, that nominate *atricapilla* has been reported (Clarke 2006). Preferred habitats show reasonably dense vegetation from sea level to higher altitudes (Martin & Lorenzo 2001, Clarke 2006).

Figure 11 Distribution of the blackcap (*Sylvia atricapilla*) in Eurasia and Northern Africa
From Shirihai et al. (2001). (1) *S. a. atricapilla*, (2) *S. a. heineken*, (3) *S. a. gularis*, (4) *S. a. pauluccii*, (5) *S. a. dammholzi*.

The spectacled warbler (*S. conspicillata*) has been divided into two subspecies (Figure 12) with *S. c. orbitalis* on Madeira, Canary Islands and Cape Verde Islands and *S. c. conspicillata* in the west Mediterranean basin and northwest Africa (Clements 2000, Shirihai et al. 2001). The species is an uncommon resident only on the main island of Madeira, where it prefers higher altitudes. On the Canary Islands it's a common resident on all islands, particularly the less vegetated eastern islands of Fuerteventura and Lanzarote. It is generally found in agricultural areas as well as low-elevation *Euphorbia* shrubs and high altitude broom (Martin & Lorenzo 2001, Clarke 2006).

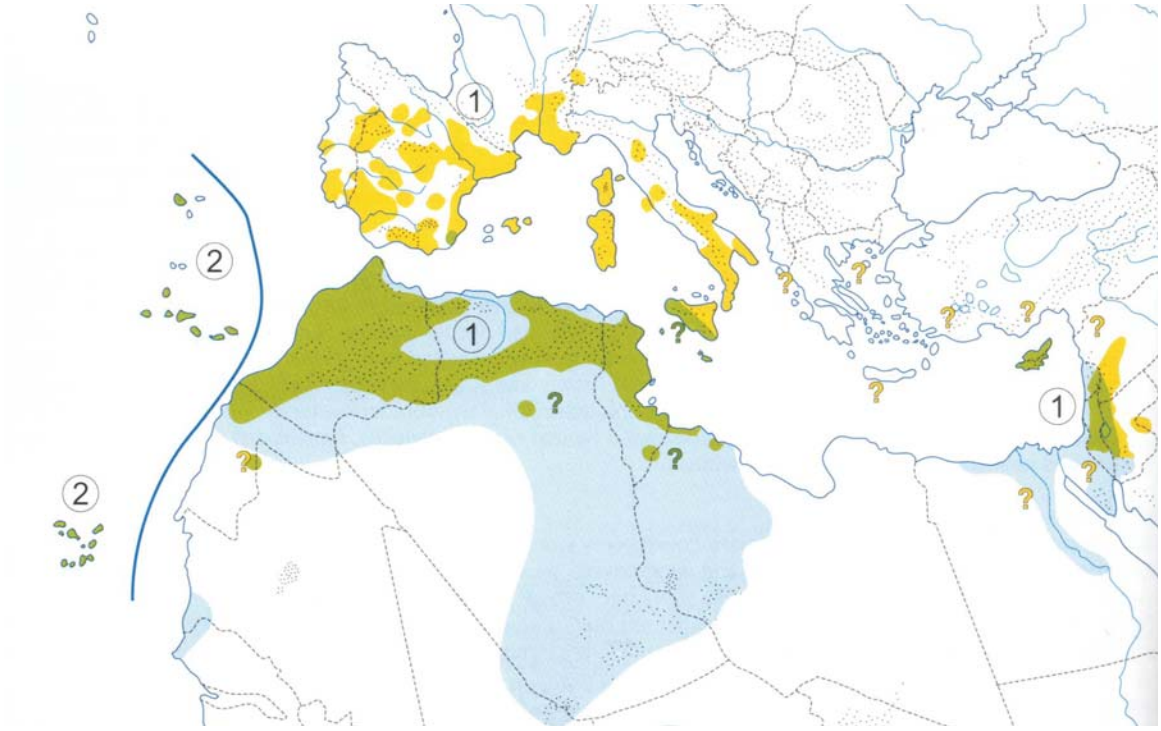

Figure 12 Distribution of the spectacled warbler (*Sylvia conspicillata*) in Europe and North Africa
From Shirihai et al. (2001). (1) *S. c. conspicillata*, (2) *S. c. orbitalis*.

1.4 Aims and scopes of the present study

Despite extensive research on the phylogeography of animal taxa on the Canary Islands (Juan et al. 2000), only little is known about the degree of differentiation and evolutionary history of birds within Macaronesia although evolutionary studies of birds on islands have a long history (Grant 2001). A first phylogeographic approach elucidated the colonization history of the chaffinch on the Canary Islands (Marshall & Baker 1999). Further molecular studies investigated the divergence between birds from the Canary Islands and the neighbouring mainland, e.g. for the chiffchaff (*Phylloscopus collybita*) (Helbig et al. 1996), Canarian stonechat (*Saxicola dacotiae*) (Wittmann et al. 1995) or Houbara bustard (*Clamydotis undulata*) (Gaucher et al. 1996, Broders et al. 2003, Idaghdour et al. 2004). Other studies used only morphological and bioacoustic characters to infer the phylogeography and (sub)specific distinctiveness of Atlantic island birds (Bannermann 1963, Grant 1979a, b, 1980, Bergmann & Schottler 2001).

Molecular markers offer a powerful tool for the investigation of inter- and intraspecific differentiation and evolution, particularly in cases of little phenotypic variation (Moritz & Hillis 1996, Cruzan & Templeton 2000). Molecular markers easily can be applied to any living organism and offer a nearly unlimited pool of variability (Avise 1994). Variation in molecular markers that are selectively neutral provides possibilities for dating divergence times, and thus allows to estimate a temporal framework for the evolutionary history of a

taxon, even if it is not or only scarcely represented in the fossil record (Cruzan & Templeton 2000). Today, a wide range of molecular markers, including mitochondrial and nuclear DNA sequences, PCR-markers, different kinds of restriction fragments (RFLP, AFLP, ISSR), mini- or microsatellites, are available for investigating the geographic pattern of genetic lineages (Avise 1994, Moritz & Hillis 1996, Avise 2000). Due to its high evolutionary rate, strictly maternal inheritance and easy handling, mitochondrial DNA (mtDNA) has been, and still is, the preferred marker system for phylogeographic studies in birds and other animals (Avise et al. 1987, Bermingham & Moritz 1998, Avise 2000).

In the present study, mitochondrial markers are analysed by phylogenetic, phylogeographic and population genetic methods and compared with morphological characters obtained from live birds. The combination of molecular and morphological data analyses should elucidate the variation and differentiation in selected passerine bird species on the Atlantic islands, and from them their evolutionary history can be inferred. Special attention is payed to the degree of differentiation within island and between island and mainland populations. For the first time it is possible, to verify or reject historic taxonomies of birds in Macaronesia by using molecular markers. Furthermore, the data presented in this study may help to identify populations of particular conservation concern on the fine geographic scale (evolutionary significant units, ESU).

The results of this thesis are presented in five independent contributions (chapter 3.1 to 3.5). Preceeding to these, background information on laboratory and analytical methods is given and general laboratory protocols are described (chapter 2).

The first contribution (chapter 3.1) describes the phylogeographic differentiation of the European robin (*Erithacus rubecula*) on the Canary Islands. The data reveal three distinct lineages, including a so far undescribed new taxon on the island of Gran Canaria (Dietzen et al. 2003).

In chapter 3.2, the phylogeography of island canary (*Serinus canaria*) populations in Macaronesia is investigated. Genetic differentiation between and within archipelagos is very low although a large number of private haplotypes confined to individual archipelagos was found. The data suggest a very recent range expansion and ongoing differentiation (Dietzen et al. 2006).

In a further study (chapter 3.3), the phylogenetic relationships and radiation of Atlantic goldcrests (*Regulus* spec.) is investigated with mitochondrial cytochrome b-data (this study) compared to mitochondrial control region and bioacoustic data (external data provided through cooperative with other institutions). The results display a strong differentiation between, and in parts also within, archipelagos, including a so far undescribed new taxon on La Palma and El Hierro (Päckert et al. 2006).

Another island radiation was found in the blue tit (*Parus teneriffae* – group) on the Canary Islands, including a new taxon from the island of Gran Canaria (Dietzen et al.

subm.). In contrast, the subspecies *degener* appears very close to birds from North Africa (*P. t. ultramarinus*). The calibration of a molecular clock allowed the comparison of evolutionary rates in the mitochondrial cytochrome b-gene (this study) and the mitochondrial control region (external data) to test the 2 %-rule (Kvist et al. 2005, Päckert et al. 2007).

The last contribution (chapter 3.5) investigates the intraspecific differentiation of members of the genus *Sylvia* in Macaronesia by comparing mitochondrial sequence data with morphological measurements. Both, the Sardinian warbler (*S. melanocephala*) and the blackcap (*S. atricapilla*), display very limited genetic variation which contradicts biometrics. The molecular data present evidence for a very recent range expansion after the last Pleistocene glaciations.

The final chapter of this thesis (chapter 4) summarizes the major findings and compares the individual contributions to each other and in a broader phylogeographic context. In the end, some aspects and starting points of future research that arise from this study are highlighted.

2 Material and Methods

2.1 Sample material

As a source of DNA, blood, tissue or feather samples were collected from living birds and road kills. Birds were caught with standard techniques using mist nets. Blood samples were taken from the brachial vein of living birds with the help of a conventional syringe, and the animals were released immediately afterwards. All samples were stored in EDTA buffer or 70% ethanol and kept at –16°C until further processing.

In total, 102 samples of *Erithacus rubecula*, 40 of *Serinus canaria*, 23 of *Regulus teneriffae*, 16 of *R. madeirensis*, 6 of *R. regulus azoricus/inermis*, 73 of *Parus caeruelus/teneriffae*, 62 of *Sylvia melanocephala*, 107 of *S. atricapilla* and 12 of *S. conspicillata* were collected. A further 517 samples of 36 other species have also been collected for possible future research projects. Since this study has kind of a preliminary character, not all samples of each species have been included in the respective contribution. Aliquots of all samples used in this study have been deposited in the Institute for Pharmacy and Molecular Biotechnology, Dept. Biology (University of Heidelberg). A list of samples with reference numbers of aliquots, detailed information on localities, GenBank accession numbers and, where available, numbers of voucher specimens is given in Appendix A. All samples have been collected with the kind permission of respective authorities on the Canary Islands (Consejería de Política Territorial y Medio Ambiente), Madeira (Região Autónoma da Madeira, Parque Natural da Madeira), the Azores (Secretaria Regional do ambiente, Direccão de Serviços da Conservação da Natureza) and in Morocco (Department des Eaux et Forests, Royaume du Maroc). Further samples were provided by S. Leitner, Vogelwarte Radolfzell (*Serinus canaria*), H.-H. Witt (several species), E. Garcia-del-Rey, Universidad de la Laguna, and G. Delgado Castro, Museo de Ciencias Naturales Santa Cruz de Tenerife (*Sylvia melanocephala, S. conspicillata, Parus teneriffae*). Additional sequences of investigated species were available in GenBank. For outgroup rooting, sequences of closely related taxa were taken from GenBank (Appendix A).

2.2 Equipment

All instruments that were used for laboratory analyses are listed in Table 3.

Table 3 Analytical instruments used in the present study

Instruments	Company
Automated sequencer: ABI 310, ABI 3100	Applied Biosystems
Electrophoresis microcomputer consort E452, E752	Fröbel
Gel chambers for agarose gel	Univ. Heidelberg
Laboratory scale	Sartorius
Microcentrifuge: 202 Mk	Sigma
Microcentrifuge: Biofuge Fresco	Haereus
PCR machines: TRIO-Thermoblock and T Gradient	Biometra
PH meter: MP 120 PH meter	Mettler Toledo
Pipettes: Pipetman P2, P20, P100, P1000	Gilson
UV-transilluminator II-200-M [312nm]	Bachofer
Vortex: Reax 2000	Heidolph

2.3 Solutions and chemicals

A list of chemicals, enzymes and other materials used in this study is given in Table 4, followed by a list of buffers and solutions in Table 5.

Table 4 Chemicals, enzymes and solutions used in this study

Chemicals, Enzymes and other Materials	Company
Acetic acid	Merck
Agarose	HYBAID-AGS
Ammonium sulfate	Gerbu
Big Dye Terminator kit	Applied Biosystems
Bovine serum albumin (BSA)	Sigma
Chloroform	Fluka
EDTA	Roth
Ethanol absolute	Sigma
Ethidium bromide	Serva
Formamide	Applied Biosystems
Guanidine thiocyanate	Roth
Isopropanol	Applichem
β-mercaptoethanol	Merck
Nucleotides	Sigma
Phenol	Merck
Proteinase K	Merck
Reaction tubes (0.2, 0.5, 1.5, 2 ml)	Eppendorf
REDTaq^{TM} DNA polymerase	Sigma
Sequagel Solution	Biozym
Sodium dodecyl sulfate (SDS)	Applichem
Sodium acetate	Merck
Sterile filter, 0.45 µm	Sartorius
Taq DNA polymerase	Sigma
Tris-HCl	Roth

Table 5 Buffers and solutions used in this study

Stock Solutions	
Agarose gel solution	1% agarose, 1 µg/100 ml ethidium bromide, in 1x TAE
Ammonium acetate	4 M ammonium acetate, in water
Ammonium persulfate	10% solution in water
Chloroform/isoamyl alcohol	Chloroform, isoamyl alcohol in a ratio 24:1
EDTA buffer	10% EDTA, 0.5% NaF, 0.5% thymol, 1% Tris (pH 7.5)
Guanidine thiocyanate buffer	4 M guanidine thiocyanate, 0.1 M Tris-HCl, 1% β-mercaptoethanol, pH 5
λ-PST I size standard	λ-DNA cut with PST I restriction enzyme
Lysis buffer	25 mM EDTA, 75 mM NaCl, 10 mM Tris-HCl, pH 7.0
Nucleotide mix	2.5 mM dATP, 2.5 mM dCTP, 2.5 mM dGTP, 2.5 mM dTTP,
PCR buffer (10X)	100 mM Tris, 500 mM KCl, 5% Triton X-100, 15 mM $MgCl_2$, hydrochloric acid (pH 8.5)
Phenol/chloroform	Phenol, chloroform, isoamyl alcohol in a ratio 25:24:1, 1 g 8-Hydroxychinolin
SDS solution	20% solution in water
Sodium acetate solution	3 M sodium acetate, acetic acid (pH 4.6)
Sodium chloride solution	Sodium chloride in water (saturated)
TAE buffer	40 mM Tris, 1 mM EDTA, acetic acid (pH 8.0)
TBE buffer (10X)	1 M Tris, 89 mM boric acid, 10 mM EDTA, pH 8.6
TE buffer	10 mM Tris, 1 mM EDTA, hydrochloric acid (pH 8.0)

2.4 DNA isolation

Isolation of total genomic DNA followed standard protocols (Sambrook & Russell 2001). Small aliquots of sample material were digested for several hours at 50°C in lysis buffer in the presence of 1% SDS and 1 mg of proteinase K. Cell fragments and proteins were precipitated with saturated NaCl solution and subsequent centrifugation or by standard phenol/chloroform extraction. The DNA was precipitated from the supernatant by adding 0.8 Vol.-% of ice-cold isopropanol. The extracted DNA was washed twice with 70% ethanol, dried and redissolved in TE buffer. DNA stock solutions were kept at 4°C until further analysis.

In cases of very limited sample material and low DNA yield, the protein pellet was redigested by addition of guanidine isothiocyanate buffer to extract DNA that was trapped in the pellet. In this case, digestion was followed by extraction twice with phenol/chloroform, then once with chloroform/isoamyl alcohol and subsequent precipitation and washing of the DNA as explained above.

To determine the approximate concentration and quality of the extracted DNA, 3 µl of each DNA solution were loaded onto a 1% agarose gel containing ethidium bromide. DNA

concentration was estimated by comparison of fluorescence intensities to samples of known DNA content.

2.5 Sequencing of mitochondrial genes

2.5.1 The use of mitochondrial DNA in phylogeographic studies

More than half of all studies on animal phylogeography use mitochondrial DNA (mtDNA) as a genetic marker (Avise 2000). The animal mitochondrial genome seems particularly useful for phylogenetic and phylogeographic analyses due to a number of characteristic properties. These are its ubiquitous distribution, ease of isolation and manipulation, simple genetic structure, simple mode of inheritance and fast evolutionary rate (Avise et al. 1987, Moritz 1994).

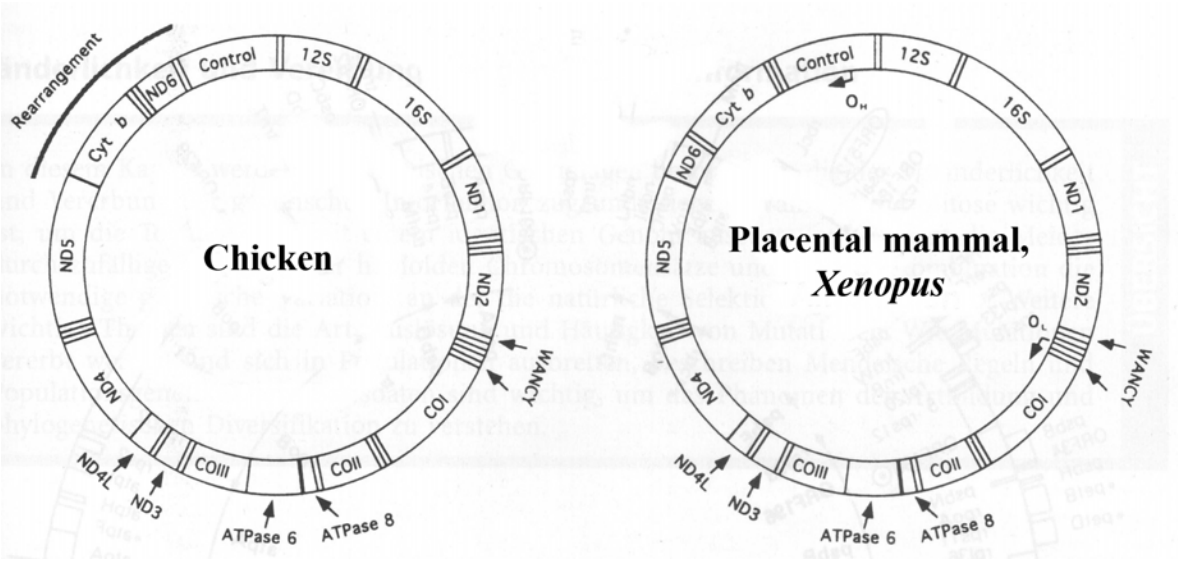

Figure 13 Gene content and order of the mitochondrial genome of birds (Desjardins & Morais 1990) compared to mammals and amphibians (Quinn 1997)

In birds, mtDNA is a small, circular molecule, about 16 kilobases long (Desjardins & Morais 1990). It contains genes for 13 proteins, 2 rRNAs and 22 tRNAs and an additional non-coding region, the so-called '*control region*', which controls mtDNA replication. Although it contains the same genes as all other vertebrates, the order of these genes is unique (Figure 13). Several studies have shown that this altered gene order is conserved across a wide taxonomic range of birds, including ducks, geese, dunlin, turnstone, murre, gulls, sandpipers (Quinn 1997), while further bird-specific rearrangements have been found in other groups (Boore 1999, Bensch & Härlid 2000). While tRNA rearrangements have been observed in other vertebrates, the avian and lamprey genomes are thus far the only ones known to have undergone major reaarangements that include protein-coding genes (Quinn 1997). It is assumed that tandem duplication of part of the mitochondrial

genome followed by deletions is the mechanism underlying these rearrangements (Moritz et al. 1987, Quinn 1997). The comparison of the avian mitochondrial genome to those of mammals and amphibians shows similarities besides the common gene content (Desjardins & Morais 1990, Quinn 1997): (1) several genes end with incomplete stop-codons, (2) codon/anticodon rules are the same, (3) guanine is relatively infrequent at third codon positions, (4) several genes, including ATPase 6 and 8, overlap by the same amount as in *Xenopus* but less than in mammals, (5) the control region includes a transcriptional promoter, which, as in amphibians, is bidirectional. Unique features found in the mitochondrial genome of birds only are the lack of the hairpin structure that forms the light strand origin of replication, which is located between $tRNA^{Asn}$ and $tRNA^{Cys}$ in amphibians and mammals. In chicken and goose the COI has a putative GTG initiation codon that is unusual among vertebrates and is unique for this gene. Another feature peculiar for birds is the low incidence of thymine at silent positions within coding regions of cytochrome b and presumably other protein-coding genes coded on the same strand (Desjardins & Morais 1990, Quinn 1997). The extrem compositional bias created by the thymine and guanine deficit may make saturation effects particularly severe in DNA sequence-based phylogenetic studies of birds (Kocher et al. 1989).

In the present study, the cytochrome b gene and small fragments of adjacent ND5 gene and $tRNA^{Thr}$ were sequenced. These genes encode mRNAs specifying polypeptide units of proteins involved in electron transport and oxidative phosphorylation that take place on the inner membrane of the mitochondrion.

Whereas the gene order is relatively conserved, the mutation rate within the genes is high. Evolutionary rates of mtDNA in animals have been estimated to be at least ten times faster than the average evolutionary rate of the nuclear genome (Graur & Li 2000). A first approximization of the evolutionary rate in mtDNA has been proposed by (Brown et al. 1979). They suggested an average rate of 2% sequence divergence between two taxa that evolved independently for one million years. Further peculiarities of animal mtDNA refer to deviations from the “universal” genetic code and to an unequal base composition which is reflected in a strong bias against G on the L-strand (Desjardins & Morais 1990, Doadrio et al. 2002).

Inheritance of animal mtDNA is strictly maternal. Therefore, mtDNA often shows clearer patterns of geographic differentiation than the recombining nuclear DNA. However, this means also that mtDNA reflects only the maternal lineage of inheritance, which can lead to false inferences of phylogeographic patterns, especially in species with sex-biased dispersal (Moritz 1994).

2.5.2 Laboratory protocols for sequencing of mitochondrial marker genes

Amplification of target fragments

One mitochondrial marker gene was sequenced in this study, the cytochrome b gene. Depending on primer position in some cases parts of adjacent genes were also included. Polymerase chain reactions (PCR) with specific primers situated in the flanking regions of the target gene (Table 6) were performed to amplify the fragments of interest. To sequence the cytochrome b gene of highly degraded DNA, additional primers situated within the cytochrome b gene were used for amplification of short fragments (Table 6).

PCR was performed in 50 µl volume containing 0.75 units of *Taq* polymerase, 0.2 mM of each dNTP, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 % Triton x-100, and 10 mM Tris-HCl (pH 8.5). 10 pmol of each primer and 50-100 ng of the template DNA were used. PCR reactions were performed in a Biometra thermoblock according to the following temperature profile. For some difficult samples this profile was slightly modified with regard to annealing temperature or number of cycles.

94°C	10 min	
94°C	60 sec	|
53°C	60 sec	| 30 cycles
72°C	2 min	|
72°C	10 min	
4°C	∞	

Prior to further analysis, the success of the PCR was checked on 1 % agarose gels to which a size standard (λ-DNA cut with PST I restriction enzyme) was loaded for size comparison. Where necessary, a re-amplification of the PCR product was performed under the same conditions as above but with 1 µl of the 1:10 diluted initial product as template DNA.

Table 6 **Primers used for amplification and sequencing of the mitochondrial cytochrome b gene**
Indicated are the sequences of each primer, used for amplification (amp.) and/ or sequencing (seq.), location on the L- or H-strand, position of the 3' nucleotide within the mitochondrial genome of a reference sequence of the chicken *Gallus domesticus* (Desjardins & Morais 1990).

Primer	Sequence	Use	Position[1]
L14850	5'-TAC CTG GGK TCT TTC GCC C-3'	amp., seq.	ND5
mt-A1 (L14995)	5'-GCC CCA TCC AAC ATC TCA GCATGATGAAAC TTC CG-3'	amp., seq.	Cyt b
mt-FS-H (H15917)	5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3'	amp.	Cyt b
L14464	5'-CTW GGC AGC ATT AYA GCA GG- 3'	amp., seq.	ND5
L14854	5'-GGK TCT TTC GCC CTM TC-3'	amp., seq.	ND5
mt-c (L15320)	5'-TAY GTC CTA CCA TGA GGA CAA ATA TCA TTC TGA GG-3'	seq.	Cyt b
mt-e (H15700)	5'-GAT GGC GTA GGC AAA TAG GAA GTA TCA TTC TGG TTT-3'	seq.	Cyt b

[1] Position of the 3' nucleotide of the primer in the mitochondrial genome of the chicken *Gallus domesticus* (Desjardins & Morais 1990).

Sequencing

PCR products were sequenced directly on automated sequencers with the primers listed in Table 6. Sequencing on ABI Prism 310 and ABI Prism 3100 (Applied Biosystems, Amsterdam) was performed with the Big Dye Terminator kit (vers. 1.1, 2.0 and 3.1) following the manufacturers' instructions. The reactions were run in a Biometra thermoblock under the following temperature profiles.

ABI 310, ABI 3100 and MegaBase 1000:

96°C	10 sec	|
52°C	5 sec	| 25 cycles
60°C	4 min	|
4°C	∞	

2.5.3 Data preparation

The obtained sequences were aligned manually. To check for sequence errors, all sequences from closely related populations were carefully compared and all variable sites extracted with the program package MEGA vers. 2.1 (Kumar et al. 2001) were checked individually in the sequence printouts. Further, all sequences were checked for unexpected stop codons.

All sequences obtained an open reading frame without unexpected stop codons and a strong bias against guanidine on the L-strand, as is typical for mitochondrial DNA (see above). It therefore may be inferred that the sequences represent the functional genes rather than nuclear pseudogenes (Zhang & Hewitt 1996, Bensasson et al. 2001).

2.6 Data analysis

In the following paragraphs, the methods used for data analysis will be introduced, by giving background information and a rough outline of the procedure. More detailed information on data analysis, like parameter settings etc., will be given in the particular chapters.

2.6.1 Reconstruction of phylogenetic trees

Many techniques have been described to infer phylogenetic trees from all types of molecular and other data. The selection of an appropriate method depends on the question to be addressed, the type and quality of the data and – due to the complexity of some methods – the availability of computation resources.

Inferring a phylogeny is an estimation procedure based on the information contained in the data. As we usually have access only to contemporary species and molecules but not to direct information about the past, it is most critical to have some basis for selecting one or more preferred trees from the large set of possible phylogenies. There are two ways of doing this. First, by defining a specific algorithm that directly leads to the determination of a preferred tree, and second, by defining an optimality criterion according to which the "best tree" can be selected from all possible trees (Swofford et al. 1996). A third and relatively new but increasingly utilized approach, uses Bayesian inference for reconstruction of phylogenetic trees (Yang & Rannala 1997, Hall 2001, Huelsenbeck & Ronquist 2001).

Distance methods

Purely algorithmic methods include all forms of pair-group cluster analyses as well as some other distance methods (e.g. neighbor joining, UPGMA). Here the neighbor joining algorithm (Saitou & Nei 1987) as implemented in PAUP vers. 4.0b10 (Swofford 2001) is used to infer phylogenies from cytochrome b sequence data. Neighbor joining is related to traditional cluster analysis, but removes the assumption that the data are ultrametric (Swofford et al. 1996).

Distance methods infer the relationship between taxa from a specified matrix of pairwise distances. Such distances may represent absolute or proportional numbers of differences between pairs of sequences or genotypic data, or they can be calculated based on particular evolutionary models. For example, the Kimura-2-parameter distance (Kimura 1980) takes into account the different rates at which transitions and transversions occur.

Criterion-based methods

The criterion-based methods include those known as maximum parsimony and maximum likelihood. They have two logical steps, the first is to define an optimality criterion, which is formally described by an objective function, and the second is to use specific algorithms for computing the value of the objective function and for finding the tree(s) that have the best values according to this criterion (Swofford et al. 1996).

The optimal tree under the parsimony criterion is the tree that requires the fewest number of character-state changes. Parsimony methods therefore operate by selecting trees that minimize the total tree length. If it is desirable to take into account that not all characters are equally informative or reliable with respect to the evolutionary history of the taxa under study, characters may be weighted. One frequently used case of character weighting in sequence data refers to the down-weighting of the third codon position in protein-coding genes, when this position is saturated (see below).

The maximum likelihood criterion evaluates the probability that a proposed model of an evolutionary process and a hypothesized history (represented by the phylogenetic tree) would give rise to the observed data. This approach requires the definition of an appropriate evolutionary model that includes information e.g. on rate parameters of the substitution matrix and parameters used in modelling rate heterogeneity among sites (Swofford et al. 1996). The values for these parameters can either be supplied on the basis of extrinsic evidence or be estimated from the data. To choose the model that best fits the data, likelihood ratio tests may be performed on alternative models. This approach is implemented in the program MODELTEST vers. 3.06 (Posada & Crandall 1998). This program runs a series of likelihood ratio tests on a number of pre-specified increasingly complicated models and chooses the appropriate model to which addition of further parameters does not result in significantly better likelihood scores (Posada & Crandall 1998).

Searching for the optimal tree is usually limited by computation resources. For large data sets, it is therefore necessary to use heuristic search approaches. These sacrifice the guarantee of optimality in favour of reduced computing time, as they may “get trapped in a local optimum instead of reaching the global optimum” of the tree distribution (Swofford et al. 1996). One way to reduce the probability of ending up in a local optimum is to perform post-analytical branch-swapping. In branch-swapping, a set of predefined rearrangements is performed on the selected tree topology. This might lead to formerly undetected topologies that have better values according to the applied optimality criterion. The most widely used algorithm for branch-swapping is the ‘tree-bisection-and-reconnection’ algorithm. In this approach the tree is bisected along one branch, and the two disjoint subtrees are then reconnected by joining a pair of different branches. All possible bisections and pairwise reconnections are evaluated during branch-swapping.

In cases where more than one optimal tree is found, a consensus tree is calculated from the rival trees. Strict consensus trees contain only those groups that appear in all best trees. Majority-rule consensus trees preserve all compatible groups that occur on a certain pre-specified percentage of the rival trees. Phylogenetic reconstructions under criterion-based approaches have been performed using PAUP vers. 4.0b10 (Swofford 2001).

Assessing the reliability of an inferred phylogeny

Usually there is no evidence about the real evolutionary scenario. It therefore seems desirable to objectively assess the reliability of an inferred phylogeny.

To assess the clarity of a single data set, tree topologies may be calculated under various algorithms and criteria and the outcomes of the different analyses may be compared. The more concordant the results are, the better support exists that the information contained in the data favours a single evolutionary history and that this is actually reflected by the inferred phylogeny. Furthermore, several indices have been proposed to infer the reliability of a phylogeny, e.g. the consistency index (CI), homoplasy index (HI) and retention index (RI). CI and HI are estimates for the number of homoplasies as a proportion of all character state changes in a topology. CI defines the proportion of character state changes that is not attributable to homoplasies, and HI = 1 – CI defines the proportion of changes that is attributable to homoplasies. The closer CI to 1, the better the agreement between topology and data set (Wägele 2000). RI measures the proportion of potential synapomorphies. The more characters show unambiguous distribution of states within the taxa (i.e. no homoplasies, but only synapomorphies), the closer RI to 1 and the more reliable the phylogeny (Wägele 2000).

To assess the reliability of individual branches, the bootstrap (Felsenstein 1985), a nonparametric resampling method, is probably the most widely used approach. By bootstrapping, a series of pseudoreplicates is created from the original data set and used for phylogenetic reconstruction. The proportion of pseudosamples that supports a given internal branch is recorded as the respective bootstrap value.

It is important to note that if the data are not representative or if the reconstruction method makes an inappropriate estimate of the phylogeny, this bias cannot be removed by bootstrapping or any other approach of reliability testing (Swofford et al. 1996). Thus, no method of reliability testing may determine if the inferred gene topology is actually concordant with the organismal phylogeny (Nichols 2001). This can only be achieved by comparing phylogenetic reconstructions from independent data sets.

Bayesian inference

Bayesian inference of phylogeny is based upon the posterior probability of a phylogenetic tree, conditioned on the observed matrix of aligned DNA sequences and obtained using Bayes formula (Yang & Rannala 1997, Huelsenbeck & Ronquist 2001). It can be interpreted as the probability that the particular tree is the correct one under the given DNA sequence data.

Calculations were performed with the program MRBAYES vers. 3.0b4 (Huelsenbeck & Ronquist 2001). This program generates a posterior probability distribution using Markov chain Monte Carlo (MCMC) analysis under an appropriate substitution model defined by the user (Bollback 2002). The proportion of the times any single tree is found in the sample is an approximation of the posterior probability of the tree. MRBAYES uses a variant of the MCMC called Metropolis-coupled Markov chain Monte Carlo (MCMCMC) (Yang & Rannala 1997). This approach runs several chains at a time that allow a swap of the states between two chains and therefore reduces the risk to get stuck in a "local optimum" of the probability distribution.

The analysis is usually run for at least one million generations with every 100^{th} or 1000^{th} tree being sampled. A 'burnin' is determined as the point in the chains, when the log-likelihood values reach an asymptote over a large number of generations. At this time, the estimated parameters should have reached stationarity. Posterior clade probabilities for each branch are then calculated using the trees visited by the Markov chains after 'burnin'. Therefore, Bayesian inference not only reconstructs a phylogeny but generates support values for all monophyletic groups within the topology at the same time.

Rooting of a tree

To infer the evolutionary direction of a given topology, one must know which character states are ancestral and which are derived. This can be achieved by different approaches, namely using the outgroup comparison, midpoint rooting, evidence from ontogeny or from the fossil record (Smith 1994, Consuegra et al. 2002). In molecular data, outgroup comparison is the most frequently used approach to root a tree if no fossil molecular sequences are available. Midpoint rooting might be applied if rate uniformity can be assumed for the two most divergent lineages. Then the appropriate root is at the midpoint of the path connecting these taxa (Swofford et al. 1996).

To obtain rooted trees by the outgroup comparison, one or more outgroup taxa must be included in the data set. The ingroup portion of the tree is then rooted at the location at which the outgroup connects to the tree. The choice of the outgroup has considerable influence on the resulting tree topology (Smith 1994). Therefore, it is prudent to include more than one outgroup taxon and to choose outgroup taxa as closely related as possible to

the ingroup. However, it must be certain that the remaining taxa (the ingroup) are monophyletic with respect to the outgroup, because otherwise the tree will be rooted incorrectly (Swofford et al. 1996).

2.6.2 Molecular clock

A particular advantage of the use of molecular techniques in phylogenetic studies is the possibility to estimate divergence times of evolutionary lineages according to the concept of the molecular clock. The molecular clock hypothesis was first advanced in the 1960s (Zuckerkandl & Pauling 1965) and states that DNA and proteins evolve at an approximately uniform rate. Under this assumption, knowledge of the evolutionary rate allows to date divergence times from sequence data. However, several studies have shown that evolutionary rates exhibit various heterogeneities, e.g. across nucleotide positions within a codon, among non-homologous genes within a lineage, among classes of DNA within a genome, among genomes within an organismal lineage and among different taxonomic lineages (Avise et al. 1992). The original calibration of animal mtDNA sequence divergence between recently separated lineages of about 2 % per one million years (Brown et al. 1979) does not hold in many lineages (Avise et al. 1992). Among-lineage rate heterogeneity has been attributed to differences in population size, life-history variables, metabolic rate, generation time and DNA repair efficiency (Arbogast et al. 2002).

To apply the concept of the molecular clock, it is therefore necessary to first check the data set for constancy of the substitution rate. For this purpose, relative rate tests may be performed either on pairwise comparisons of individual taxa following the procedure of Tajima 1993), as implemented in MEGA vers. 2.1 (Kumar et al. 2001). Also, likelihood ratio tests might be performed that evaluate the differences in likelihood scores of phylogenetic reconstructions obtained with and without the assumption of a constant evolutionary rate (Arbogast et al. 2002).

For calibration of the molecular clock, it is necessary to have external evidence for dating at least one, but preferably several, events of lineage divergence. These calibration points are usually obtained from fossil evidence or paleogeographic inference.

2.6.3 Phylogeographic data analysis

Several methods have been proposed that aim to reveal which factors have influenced population structure and species divergence and how these factors interacted in space and time. At present, the most comprehensive approach to this question is probably nested clade analysis (NCA). However, the claim that NCA uses objective and quantifiable statistics in inferring historical processes (Cruzan & Templeton 2000) has been heavily criticized (Knowles & Maddison 2002). Furthermore, NCA is inexpediant for data sets

with large genetic distances between individual sequences. Therefore, the use of different analytical approaches and consideration of external evidence (e.g. paleoclimatic and geotectonic information, or results from comparative phylogeography) seem most promising at the moment to infer the evolutionary history of a species (Cruzan & Templeton 2000, Knowles & Maddison 2002).

AMOVA

Analysis of molecular variance – AMOVA (Excoffier et al. 1992) is used to quantify the percentage of variation at different levels of hierarchical subdivision and to assess the geographic pattern of population structure. By defining hierarchical groups of populations, the user provides different hypotheses of geographic subdivision. As implicated by its name, AMOVA transforms the data into an analysis of variance format, from which estimates of variance components and F-statistic analogs (Φ_{st}, Φ_{ct}, Φ_{sc}) can be derived (more information on F-statistics below). The significance of the values is tested using non-parametric permutation procedures. In this approach, the grouping that maximizes the among-group variation and proves significant is assumed to represent the most plausible geographical subdivision. AMOVA is implemented in the program ARLEQUIN vers. 2.0 (Schneider et al. 2000).

Mantel test

The Mantel test of matrix correspondence, based on the presentations by Mantel (1967), is most often used in phylogeographic analysis to address the question of 'isolation-by-distance'. Because the geographical distribution of a species is typically more extended than an individual's dispersal capacity, isolation-by-distance hypothesizes that populations in geographically close proximity are more closely related genetically than more distant populations. Mantel tests have also been used to distinguish between competing explanations of an observed pattern of genetic or geographic variation (Thorpe 1991, Thorpe et al. 1994b).

Mantel tests can be understood as an extension of regression analyses to two-dimensional variables that are presented in the form of square matrices. The partial Mantel test (Smouse et al. 1986) is an extension of the traditional Mantel test to multiple regressions. It predicts the elements of a single "response" matrix from the respective elements of more than one original matrix that are correlated. The Mantel test procedure is also implemented in the program ARLEQUIN vers. 2.0 (Schneider et al. 2000).

Pairwise mismatch distribution

Due to the effects that demography has on the amount of genetic variability maintained in a population, the present genetics of a population may provide information on past demographic events (Rogers & Harpending 1992). To assess the demographic history of a population, pairwise mismatch distributions (the frequency distribution of the numbers of differences between all pairwise haplotype comparisons in a population) has proven very useful. This distribution is usually multimodal in populations at demographic equilibrium, but unimodal in populations that have passed through a recent demographic expansion (Slatkin & Hudson 1991, Rogers & Harpending 1992). This is used to infer the statistical probability that a population has undergone a recent demographic expansion. Under the assumption that this is the case it is further possible to estimate the parameters of the expansion from the data, such as the time at which the expansion occurred, and its magnitude (Rogers & Harpending 1992, Rogers 1995, Schneider & Excoffier 1999). To calculate a mismatch distribution and to deduce the corresponding parameters of a demographic expansion, the programs ARLEQUIN vers. 2.0 (Schneider et al. 2000) and DnaSP 4.0 (Rozas & Rozas 1999, Rozas et al. 2003) were used.

Network reconstructions

Intraspecific gene evolution cannot always be adequately represented by a bifurcating tree as is customary for higher-level taxonomy. Therefore, the use of networks has been proposed to estimate intraspecific genealogies (Posada & Crandall 1998). In contrast to phylogenetic trees, networks allow for persistent ancestral nodes, multifurcations and reticulations and thus take into account different population-level phenomena. Advantages of networks over phylogenetic trees include the presence of loops that might indicate recombination or – as in the case of haplotypic data – the occurrence of reverse or parallel mutations. Furthermore, networks may imply information about the age of different haplotypes, based on the assumption that older alleles have a greater possibility of becoming interior, being more frequent in a population and are more broadly distributed geographically (Posada & Crandall 1998). Most network methods are distance methods with the common idea of minimizing the distances among haplotypes. In other cases, the likelihood function is maximized. In this study, two approaches of network estimation were used, molecular variance parsimony (Excoffier & Smouse 1994) and statistical parsimony (Templeton et al. 1992). These are implemented in the programs ARLEQUIN vers. 2.0 (Schneider et al. 2000) and TCS vers. 1.13 (Clement et al. 2000), respectively.

2.7 Morphometric measurements

Before the invention of molecular techniques and their acceptance as valuable tools for phylogenetic analyses, avian phylogenies were exclusively based on morphological (mainly comparative anatomy), acoustic and biogeographic evidence. As with molecular sequence data it is important to differentiate between phylogenetically informative apomorphies and uninformative plesiomorphies. Both morphology and molecular genetics have their advantages for systematic studies but the combination of both methods can maximize both the information content and usefulness (Hillis 1987). Diagnosability is crucial for the definition and discrimination of taxonomic units but in some cases one character set may not be sufficient to differentiate between related taxa and diagnosability is only achieved by combination of functionally independent characters (Helbig et al. 2002). When analysing morphological data it has to be considered that particular traits are influenced by environmental and/or ecological constraints (inheritable variation), which is rarely the case in molecular (heritable) data (Hillis 1987, Helbig et al. 2002), for example body size has a genetic (heritable) basis but may be modified by the environment. However, the comparison of morphological with molecular data facilitates verification of hypotheses if both character sets lead to identical results.

Since all birds were caught to obtain a small blood sample for molecular analysis, it was easy to collect some additional information such as measurements, sex and age of individual specimen. These data were collected for additional analyses of morphometrics and comparison of results with molecular data.

2.7.1 Collection of measurements

A set of 20 feather and body measurements was taken from captured birds according to standard protocols (Svensson 1992):

Wing length (maximum length, flattened and straightened wing), primary length (feather length of primary 1-9 counted from inwards), length of first secondary (counted from outwards), tarsus length (alternative method; toes bent backwards), length of bill to skull, depth of bill at feathering (bill height), bill width (at widest point, usually at feathering), distance bill tip to distal end of nostrils (NaLoSpi) and foot span as distance of tip of hind claw to tip of outer, middle and inner toe, respectively. Measurements were taken with standard rulers and calipers of the Vogelwarte Radolfzell, Germany, developed for taking bird measures. Rulers are exact to 0.5 mm and calipers to 0.1 mm. All measurements were taken by myself and are thus comparable.

Body weight: birds were weighted on a digital balance (Ohaus CS200) exact to 0.1 g.

2.7.2 Analysis of measurements

Measurements were analysed for Variance (ANOVA) using the computer program SPSS version 10.0.7 (SPSS Inc. 1989-1999). Significance levels for all statistic evaluations were

set at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***). To investigate diagnosability of populations on the basis of morphological measurements data were entered into discriminant function analyses (Wilk's Lamda) and different groupings were tested for best support. Only adult birds not in moult were included

3 Research Projects

3.1 Phylogeographic differentiation of the European robin (*Erithacus rubecula*) on the Canary Islands

3.1.1 Introduction

The European robin (*Erithacus rubecula*) is distributed over large parts of the Western Palaearctic (see also chapter 1.3.1) 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* (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 breast patch, white eye ring, grey forehead and neck-sides, 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' (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).

This study investigates the systematics of the robin (*Erithacus rubecula*) on the Canary Islands by using molecular tools. Sequences of the mitochondrial cytochrome b-gene were used to study the phylogeographic differentiation and test phylogenetic relationships of the taxa involved, in particular the validity of the specific status of the 'Tenerife robin' (*E. superbus*) as proposed by some authors (Bergmann & Schottler 2001). A further objective concerned the colonisation history of the Robin in the Macaronesian archipelagos.

3.1.2 Material and Methods

Samples

The samples for this study were obtained from live birds on the Canary Islands in 2002 (Appendix A). 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 % thymol 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 GCATGATGAAAC TTC CG-3') with mt-Fs-H (H15917; 5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3'). '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 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 CAAATA 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 (Wink et al. 2002).

Phylogenetic Analysis

By using different primer combinations, overlapping sequences with a combined length of 1 125 nucleotides were obtained. Sequences were carefully aligned and net pairwise genetic p-distances and corrected Kimura-2-parameter distances (Kimura 1980) calculated with MEGA version 2.1 (Kumar et al. 2001). Phylogenetic trees were constructed employing PAUP*4b10 - neighbourjoining and maximum parsimony (Swofford 2001) and MrBayes version 2.01, Bayesian inference of phylogeny (Huelsenbeck & Ronquist 2001). Neighbour-joining analysis was performed using Kimura's (1980) two-parameter model and bootstrapped 1 000 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 treebisection- 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 (Swofford 2001): tree length, consistency index (CI), homoplasy index (HI), retention index (RI) and rescaled consistency index (RC). Furthermore, the sequence data were analysed by using Bayesian inference of phylogeny (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 1921) and Φst 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 according to standard procedures (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 populations 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.

3.1.3 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.

1 125 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 7. 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 % (0.1–1.1 %, 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 %). 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 otherwise found on Gran Canaria only.

Table 7 **Genetic distances between populations of *Erithacus rubecula* inferred from 1 125 nucleotides of the mitochondrial cytochrome b gene**
Uncorrected genetic p-distances (below diagonal) and Kimura-2-parameter distances (above diagonal) are shown as mean net distances [%] ± s.d. In the diagonal (bold) are the within-group distances.

	[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**

The phylogenetic analysis led to more or less identical tree topologies for all three tree building methods used (see Figure 14 and Figure 15; neighbour joining results are not shown because they show a similar outcome to maximum parsimony and Bayesian inference of phylogeny). 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 Tenerife 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 cluster (82 % bootstrap) 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 %).

Φst values between robin populations from Gran Canaria, Tenerife and the western Canary Islands plus Europe are all highly significant (Table 8) and indicate a very restricted gene flow between these populations (Wright 1978). But it has to be noted that one bird caught on Tenerife is genetically more closely related to the birds from Gran Canaria (cf. Figure 14 and Figure 15). 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* vs. *rubecula*) could only explain 52.39 % of the total variance.

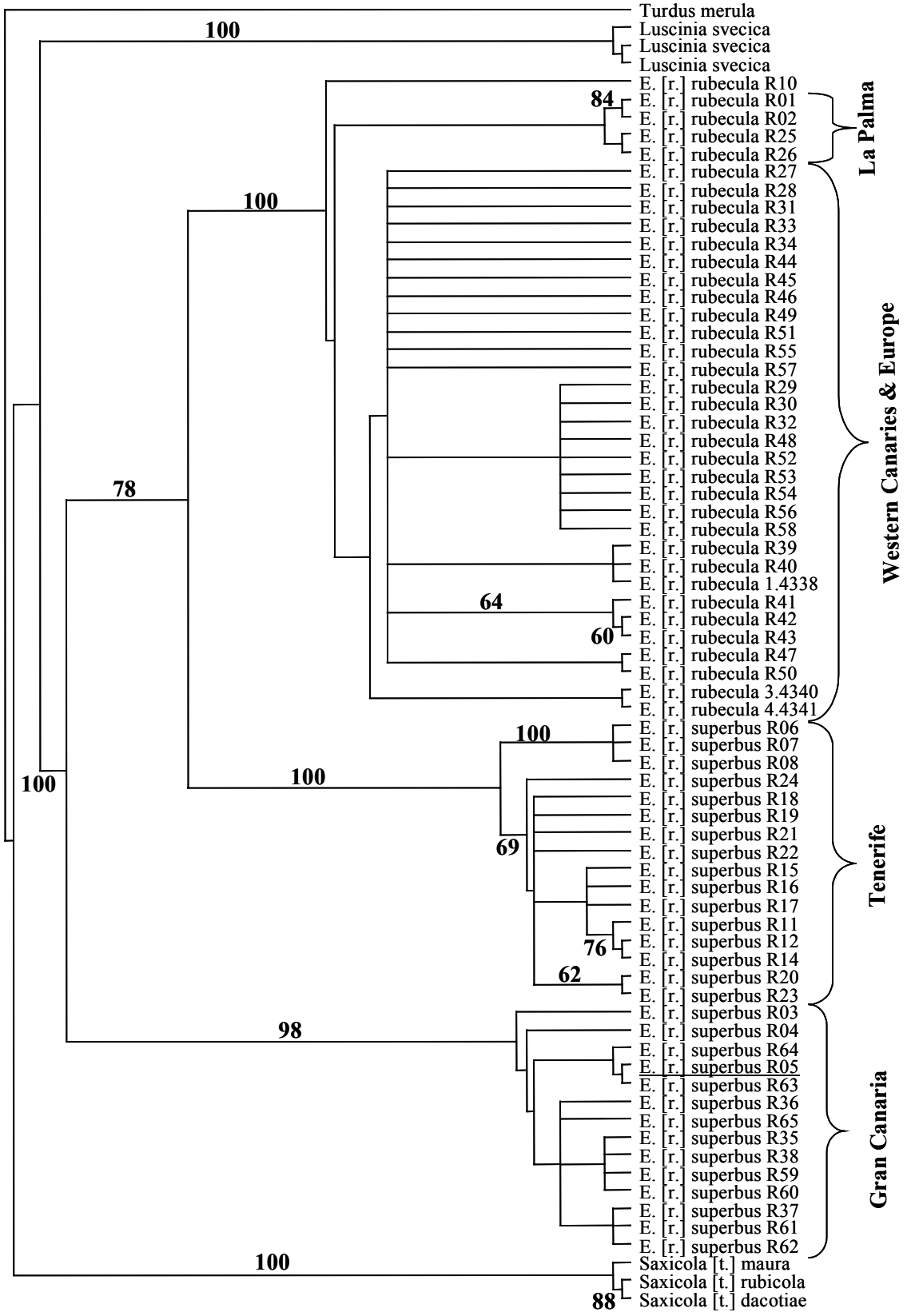

Figure 14 Maximum parsimony analysis (50 % majority rule consensus tree) of robin taxa
Numbers refer to bootstrap values above 60 % (500 replicates). Tree length 513, CI = 0.7271, HI = 0.2729, RI = 0.9176, RC = 0.6672. The underlined individual was caught on Tenerife. Samples from Madeira and the Azores cluster together with the Europe/W-Canaries clade.

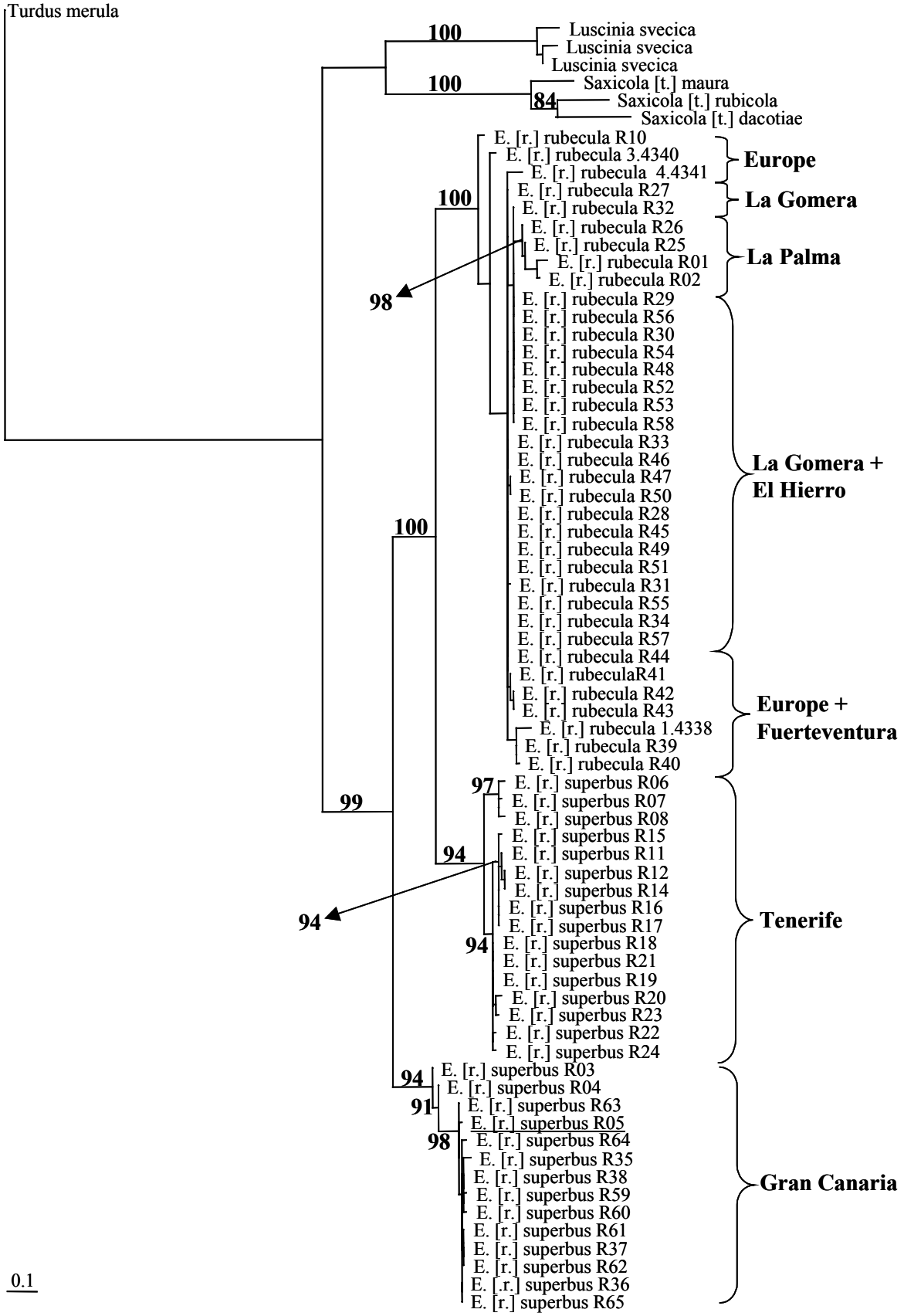

Figure 15 Bayesian inference of phylogeny of the Robin data set
Branch lengths correspond to genetic distances. The numbers indicate clade credibility values above 80. The underlined individual was caught on Tenerife.

Table 8 **Φ_{ST} values for three populations of *Erithacus rubecula* on the Canary Islands**
Significant values are indicated by * p < 0.05, ** p < 0.01 and *** p < 0.001.

Populations compared	**Φst**
Gran Canaria vs. Tenerife	0,9105***
Gran Canaria vs. Western Canaries/Europe	0,9278***
Tenerife vs. Western Canaries/Europe	0,8960***

The pairwise mismatch distribution among all individuals of the genus *Erithacus* is clearly multimodal (Figure 16a), 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 (Figure 16b; Rogers 1995). The mismatch distribution for the birds from Tenerife is multimodal indicating geographic structure or population bottlenecks (Figure 16c), but sample sizes from different parts of the island are too small to distinguish between these options. The mismatch distribution for the nominate form is rather ragged (Figure 16d) as is usually shown in populations in equilibrium (Zink 1997).

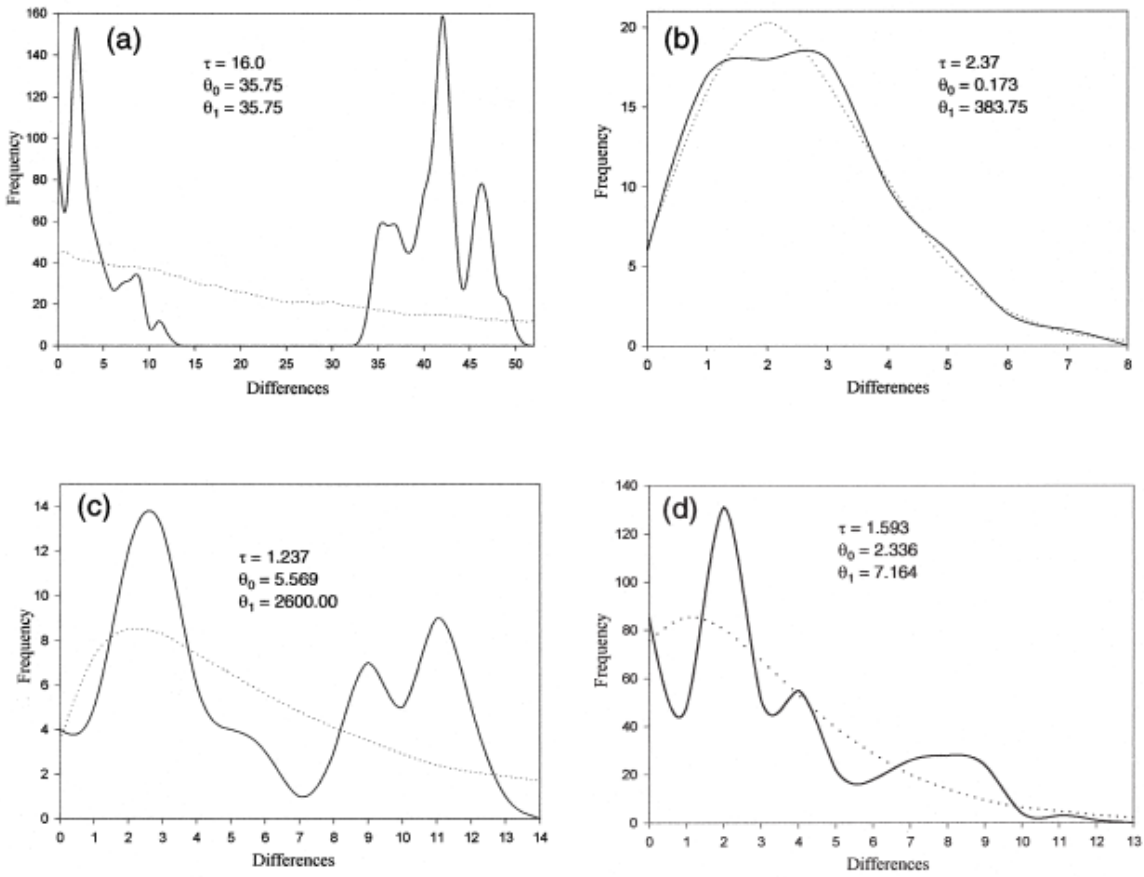

Figure 16 **Pairwise mismatch distributions**
(a) all individuals of European robins Erithacus rubecula, (b) on Gran Canaria, (c) Tenerife and (d) nominate *E. r. rubecula*. Solid lines showed the observed distribution and dotted lines the expected distribution after the 'sudden expansion' model (Rogers & Harpending 1992).

Statistical analysis of morphological measurements shows significant variance between populations, mainly due to differences in primary length and wingtip shape (Table 9). Average wing length increases obviously from Gran Canaria via Tenerife to the other islands but there is some overlap (Table 9). The mean length of P9 to P1 is shorter in birds on Gran Canaria than in those of Tenerife (Figure 17). 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 (Figure 17 and Figure 18). 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 (Figure 19). The analysis yielded three functions which explain 100 % of the variance between populations (Table 10).

Table 9 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), * ($p \leq 0.05$) or ** ($p \leq 0.01$).

Character	La Palma			La Gomera			El Hierro			Tenerife			Gran Canaria			Fuerteventura			Sign. F
	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	
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 10 Results of the Discriminant Function Analysis of measurements from *Erithacus rubecula* on the Canary Islands
Canonical discriminant function coefficients (line 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

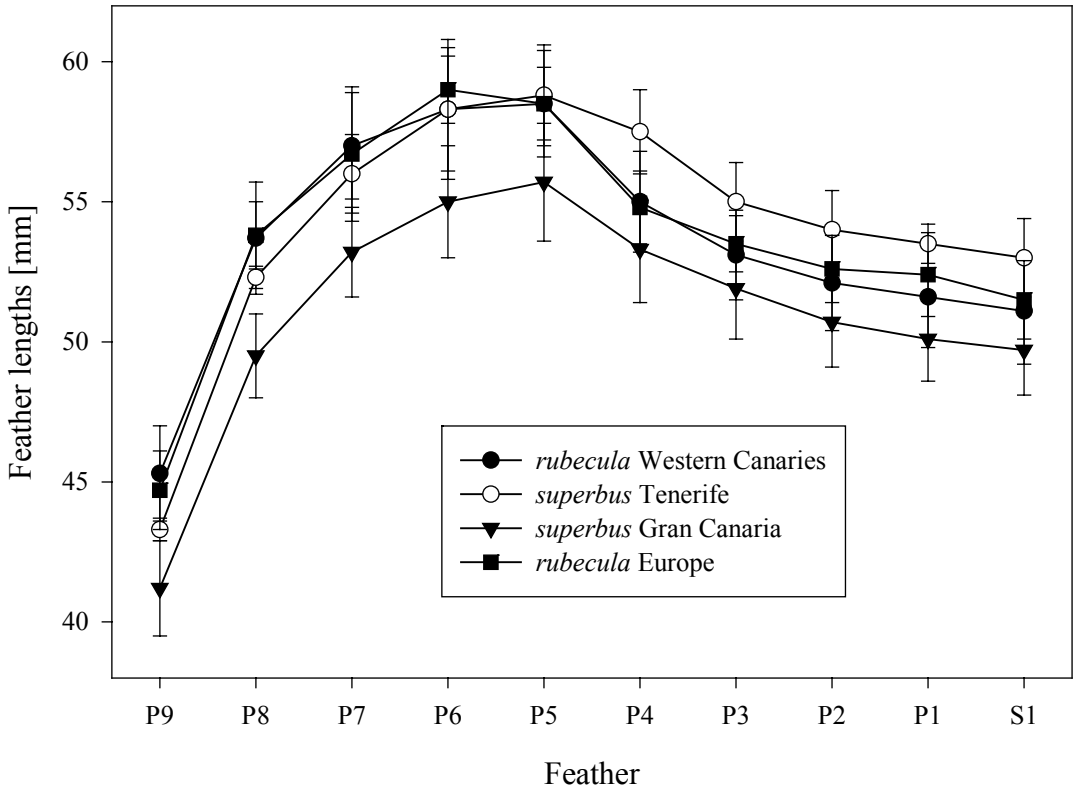

Figure 17 Wing shape of *Erithacus rubecula* on different Canary Islands and Europe (Portugal) based on measurements of primaries (P) 1-9 and secondary (S) 1

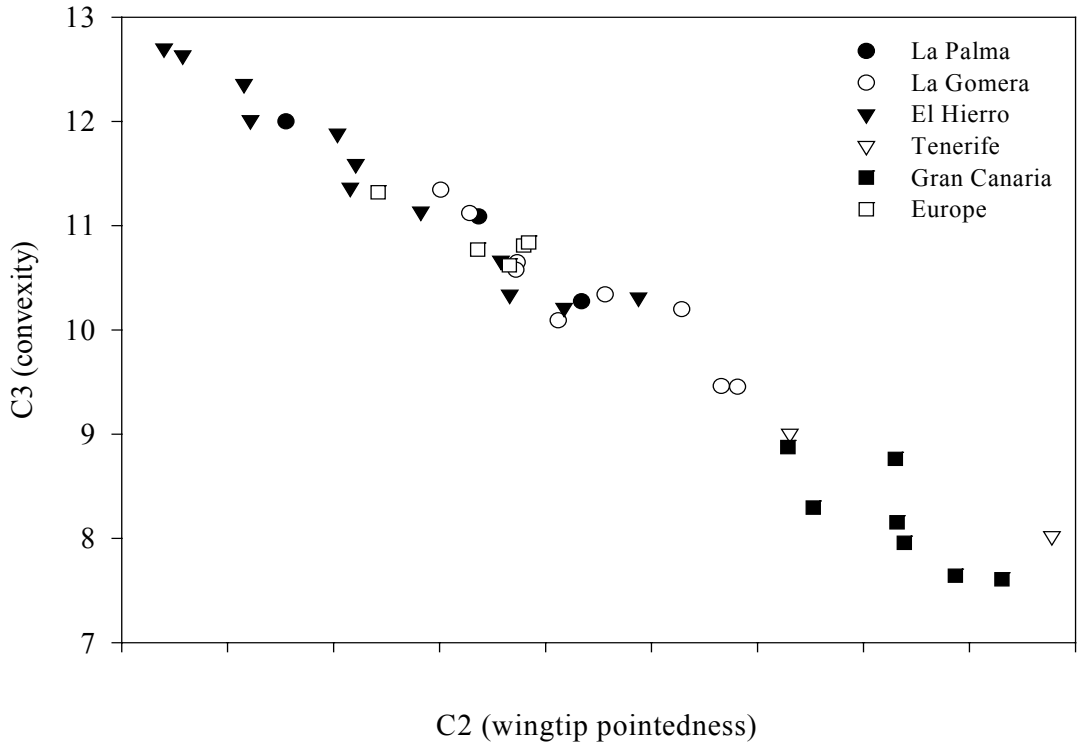

Figure 18 Wingtip shape of *Erithacus rubecula* in the Canary Islands
The two indices for characterisation of wingshape were calculated following Lockwood et al. 1998). A decrease in C2 leads to an increase in pointedness while increasing C3 leads to an increase in convexity.

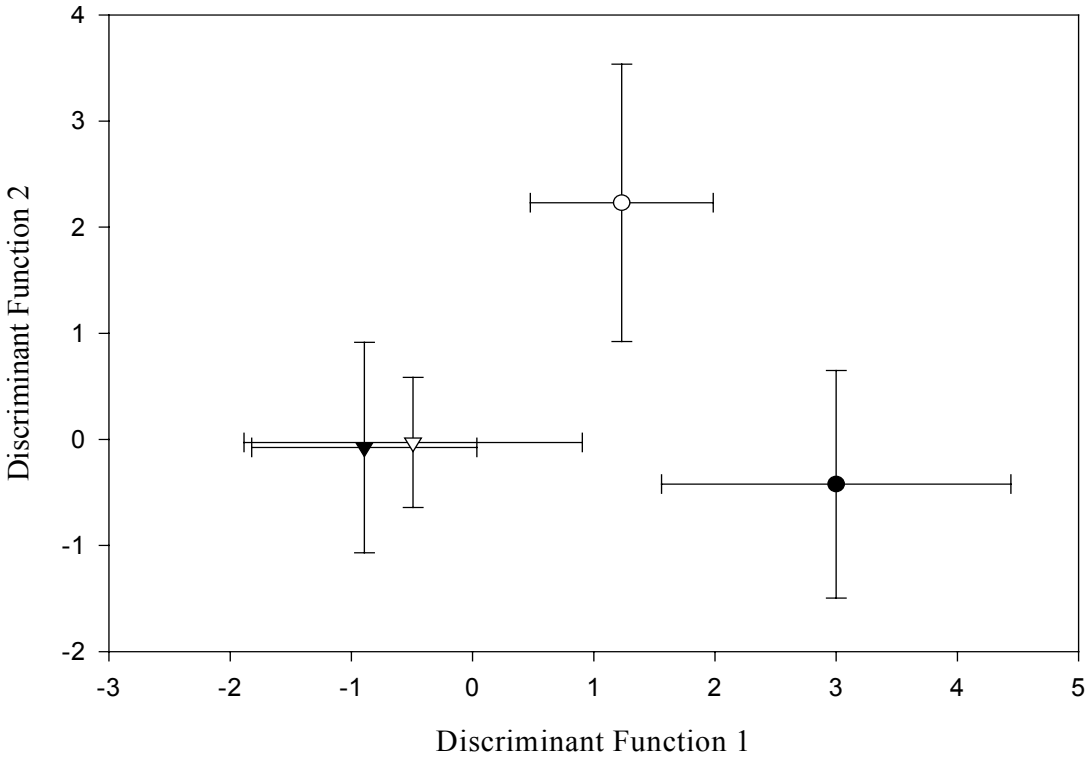

Figure 19 Plot of the first two of three discriminant functions for different populations of *Erithacus rubecula* on the Canary Islands
The group centroids with standard deviations are shown.

3.1.4 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. rubecula* (Cramp 1988) or to constitute another Macaronesian subspecies together with birds from Madeira, *E. r. microrhynchus* (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 it has to be distinguished 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 my) of those inhabited by robins today, was colonised first by a common ancestor, followed by independent colonisation of Tenerife (12 my) by the common ancestor of the Tenerife robin and *rubecula*, while the western islands (1–10 my) 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 7). 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 (Table 11). 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 Φst values, cf. Table 8) 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.

Table 11 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)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)

Examination of the pairwise mismatch distributions (Figure 16) 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 this 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, morphometrics or acoustics (Vaurie 1959, Cramp 1988, Bergmann & Schottler 2001, Martin & Lorenzo 2001). However, so far 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 measurements

indicates morphological differences between *superbus* from Tenerife and Gran Canaria, as well as between *rubecula* and both popolations 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. Figure 17 to Figure 19). 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, these results are kind of 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 accepted 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.

The 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, it is suggested keeping the Canary robins within nominate *rubecula* (Lack 1946, Cramp 1988, Clements 2000). The data presented here indicate a relatively recent colonisation of the western islands, which explains the lack of genetic and morphological differentiation. Low Φst values (not shown) indicate some gene flow 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) it is proposed to treat the taxa involved as a superspecies (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.] marionae* nov. ssp. (Gran Canaria). This genetic structuring is supported by the analysis of molecular variance.

For conservationists the 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.

3.2 Phylogeography of island canary (*Serinus canaria*) populations

3.2.1 Introduction

The genetic structure of a population reflects underlying determinants, such as gene flow, age structure and mating systems (Fleischer 1983). An important task in evolutionary biology is to elucidate the factors that determine the mating system of a population. Therefore, the knowledge of these factors will help to understand the degree of gene transmission across generations. Gene flow plays an important role in population differentiation (Bohonak 1999). Thus, different mating systems can lead to different patterns of gene flow across generations within and between populations. In the recent past, a number of studies have related breeding and mating characteristics to genetic variables. In bird communities, although about 90% of the species were categorised as having a monogamous mating system, extra-pair paternity (EPP) has been shown to be a common reproductive strategy, thereby showing enormous variation across species (Petrie & Kempenaers 1998, Wink & Dyrcz 1999, Griffith et al. 2002).

However, the diversity of mating systems is not only influenced by sexual selection alone, but also by ecological constraints. For example, island populations have been shown to have lower levels of genetic variation than those of mainlands. Generally, islands and mainlands are contrasted as opposites, while their scale is often arbitrarily applied (Grant 1998). In the comparative study of (Griffith 2000), populations are classified as being insular when the landmass is smaller than 10,000 km^2, with Gotland (Sweden) as the largest landmass to be considered as an island, and Britain as the smallest landmass classified as mainland. When assuming that the relationship between population size and area of landmass is a continuous scale, then it is possible to compare large islands with smaller ones in the same way that island–mainland comparisons have been conducted. This is particularly useful in species that live exclusively on islands, such as the Darwin's finches on the Galapagos islands (Lack 1947, Grant 1986). These finches comprise a group of passerine birds that have been well described over several decades in terms of their morphology, behaviour and ecology, but their phylogenetic relationship was only elucidated very recently (Sato et al. 1999).

In contrast, island canaries (*Serinus canaria*) have not been studied systematically in their natural habitats, although they are well known from laboratory studies of their domesticated form (Leitner et al. 2001). Recently, their breeding biology was investigated in more detail on a small island of the Madeiran archipelago (Voigt & Leitner 1998, Leitner et al. 2003). Moreover, in a parentage analysis on island canaries, no evidence for EPP was found, which is believed to be explained by ecological and non-genetic characteristics (Voigt et al. 2003). In order to determine the genetic and phylogeographic differentiation of this species, it is important to investigate the genetic structure of different populations that are geographically separated. Island canaries live exclusively on islands

within the group of Macaronesia in the North Atlantic Ocean (Azores, Madeira, Canary Islands). These islands range in size from about 2 400 km^2 (Tenerife, Canary Islands) to small islands such as Ilheu Chão (Madeiran Archipelago) which is only 0.5 km^2 in size. The aim of this contribution was to conduct a phylogeographic study on several Macaronesian islands based on nucleotide sequences of the mitochondrial cytochrome b-gene, which has been successfully used as genetic marker in previous inter- and intraspecific phylogenetic and phylogeographic studies in passerine birds (Arnaiz-Villena et al. 1998, Arnaiz-Villena et al. 1999, Arnaiz-Villena et al. 2001, Ericson et al. 2002, Irestedt 2002, Marks et al. 2002, Salzburger et al. 2002b, Salzburger et al. 2002a, Weibel & Moore 2002, Dietzen et al. 2003, Ericson & Johansson 2003, Päckert et al. 2006).

Therefore, in the island canary it is of particular interest: (1) if the geographically isolated archipelagos of the Azores, Madeira and Canary Islands are already promoting a genetic differentiation between populations, as a starting point for further investigations of the phylogeography and colonization history, and (2) in comparing the island canary cytochrome b data with morphological measurements to estimate the degree of differentiation within these island populations. This study will provide a basis for further analyses regarding the influence of mating systems on genetic differentiation and evolutionary processes on islands in general.

3.2.2 Material and Methods

Field work

Data were collected on Madeira (Ponta do Pargo: 32°49'N, 17°17'W and Santana: 32°48'N, 16°54'W) and on Ilheu Chão (32°35'N, 16°32'W), both Madeiran Archipelago, from 1995 to 1999, as well as on Pico, Azores (Candelaria: 38°28'N, 28°31'W and Serra: 38°30'N, 28°20'W) and on some of the Canary Islands (El Hierro, La Gomera, Tenerife, Gran Canaria, Fuerteventura, Lanzarote: 27°42'N, 18°01'W – 29°02'N, 13°38'W) in 2002 (Appendix A). Birds were captured with mist-nets and each individual received a unique combination of a numbered aluminium ring and two plastic rings (except on the Canary Islands where birds have not been banded). Immediately upon capture, a blood sample (approximately 100 µl) was taken from the wing vein and stored either in Queens lysis buffer, storage buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M sodium–EDTA, 1% lauroylsarcosine, pH 8.0) or in 100% ethanol at –20°C until analysis. Morphological measurements such as wing length, beak length and body weight were conducted by the same person (S. Leitner) using standard methods following a protocol of the bird banding station at Vogelwarte Radolfzell (Germany). Measurements were exact to 0.5 mm (wing length) and 0.1 mm (beak length, from feathers). Body weight was measured using a Pesola spring balance (Pesola, Baar, Switzerland) with an accuracy of 0.25 g.

Molecular genetics

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. Polymerase chain reaction (PCR) was used to amplify a fragment containing the target sequence (1 143 nt of the mitochondrial cytochrome b gene) as described earlier (Leisler et al. 1997, Broders et al. 2003, Dietzen et al. 2003).

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'), and 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'). '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 pmol of the above primers. The PCR 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 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 with 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- and 80-cm capillaries and POP6 as a polymer.

Phylogenetic analysis

By using different primer combinations, overlapping sequences with a combined length of up to 1 113 nucleotides were obtained. As an outgroup, published sequences from Genbank were used: two Eurasian Serins (*Serinus serinus*) (L76263, L76266) and one Yellow-fronted Serin (*S. mozambicus*) (L76265). Sequences were carefully aligned. For a 1 000-nt data set which was complete for all individuals, net pairwise genetic p-distances and corrected Tamura & Nei (1993) distances were calculated. A minimum spanning network was constructed employing TCS 1.13 (Clement et al. 2000). Analysis of molecular variance (AMOVA) using genetic distances and haplotype frequencies was calculated with Arlequin v.2.0 (Excoffier et al. 1992). For the AMOVA, the sequences were grouped according to larger geographic regions, i.e. Madeiran Archipelago, Canary Islands and Azorean Archipelago. The demographic history and related values were estimated via pairwise mismatch distribution using DnaSP v.3.51 (Rozas & Rozas 1999).

An appropriate substitution model for the molecular dataset was estimated via likelihood ratio test with Modeltest 3.04 (Posada & Crandall 1998). The selected model was the Tamura–Nei model, TRN+G (Tamura & Nei 1993). Likelihood settings were as follows: empirical base frequencies pA = 0.2927, pC = 0.3307, pG = 0.1316, pT = 0.2450; substitution rates R = 1 except $R_{[A–G]}$ = 1.7239, $R_{[C–T]}$ = 6.3665; gamma distribution shape parameter $\alpha = 0.1983$. For phylogenetic and phylogeographic analyses, genetic distance values were compared from individuals on the Azores (Pico) (n = 14), Madeira (n = 11), Ilheu Chão (n = 30) and Canary Islands (n = 10). Only adult males and females were used for morphological measurements that were available from the Azores (Pico) (n = 28), Madeira (n = 28) and Ilheu Chão (n = 205) (Appendix A). Genetic distance data were compared by means of a Kruskal–Wallis ANOVA and morphological data by means of a two-way ANOVA with population and sex as factors following post hoc tests using StatView 5.0 software. Bonferroni correction was applied on multiple morphological measurements and α' was set at 0.017 for all comparisons.

3.2.3 Results

Genetic distances

A complete fragment of 1 000 nucleotides of the mitochondrial cytochrome b gene was sequenced from 65 island canaries. The island canary dataset comprised 22 different haplotypes (haplotype diversity, $\hat{h}$ = 0.678; nucleotide diversity, p = 0.00190) with 38 variable sites of which 30 were parsimony informative. The nucleotide and haplotype diversity was highest within the Azorean birds (p = 0.00420, $\hat{h}$ = 0.94505), the highest theta value was also found there ($\theta = 0.00775$). The lowest diversity values were found on Madeira (Table 12). The pairwise genetic p-distances and the Tamura–Nei distances both

within and between different island canary populations were very small and individual values ranged between 0 and 1.1% (see Table 13 for mean values). In some cases genetic distances of individual birds within a population were even larger than from individuals between different populations. However, significant differences between populations were found when comparing their intraspecific genetic distance values ($H = 71.04$, $P < 0.0001$). The population from Pico (Azores) differed significantly from the populations of Madeira and Ilheu Chão ($P < 0.001$) and also from the individuals of the Canary Islands ($P < 0.05$). Moreover, the population of Madeira was different from that of the Canary Islands ($P < 0.05$). The overall mean genetic Tamura–Nei distance for all *S. canaria* included in this study was 0.37 ± 0.04%.

Table 12 DNA polymorphism in the studied populations of Island Canary (*Serinus canaria)*
π = nucleotide diversity, $\hat{h}$ = haplotype diversity, $\theta = 4N\mu$ with N describing the effective population size and μ the mutation rate per gene, Tajima's D tests for deviations from expected values. * samples from Ilhéu Chão are included in Madeira for this analysis.

Population	π	$\hat{h}$	θ	Number of haplotypes	Tajima's D	P for Tajima's D	N
Pico/Azores	0.00420	0.94505	0.00775	9	-1.94073	$P < 0.05$	14
Madeira*	0.00119	0.50366	0.00432	11	-2.36028	$P < 0.01$	41
Canary Islands	0.00135	0.75556	0.00181	5	-1.03527	$P > 0.10$	10

Table 13 Genetic Tamura-Nei-distances (below diagonal) and Φ_{ST} values among populations (above diagonal) between island canary populations of Pico (Azores), Ilhéu Chão, Madeira and the Canary Islands
Genetic distances are presented as mean values with standard deviations. The diagonal (bold numbers) represents mean values within the respective populations.

	Pico/Azores	Ilhéu Chão	Madeira	Canary Islands
Pico/Azores	**0.45 ± 0.10**	0.03830	0.01925	0.04563
Ilhéu Chão	0.26 ± 0.06	**0.05 ± 0.02**	0.06040	0.04278
Madeira	0.29 ± 0.06	0.09 ± 0.03	**0.11 ± 0.06**	0.07718
Canary Islands	0.33 ± 0.07	0.17 ± 0.04	0.15 ± 0.05	**0.17 ± 0.08**

The analysis of molecular variance (AMOVA) partitioned 5.22 % of the total variation between the geographical regions, 0.94 % between populations in the island groups and 93.83 % were found within the populations. The overall Φ_{ST} was only 0.06166. Φ_{SC}, which describes the variation between groups within regions was 0.00996 and Φ_{CT} as a measure for the variation of groups among regions was 0.05223. Φ_{ST} values between different populations are low (Table 13). The estimated mean per-generation number of migrants among populations N_m (Slatkin 1985) was 0.0913 indicating that gene flow is too small to override diversifying effects (Avise 2000).

Table 14 Variable characters and frequency in the cytochrome *b* haplotypes in three island canary populations

	Haplotype (*N*)	Accession number	Frequency			Variable sites			
			MD	AZ	CI	1111112 6671344789 5647716781	3334444445 0361788990 5820302571	5555556678 0144691255 5109121610	11 99999900 23488900 62819557
02_TF	A (37)	AY914098	0.71	0.21	0.50	ATCCTCACAA	CTTCTCCCCC	CACCACTCTA	ATTCTTCT
03_HI	B (2)	AY914099	–	–	0.20	....	....	....T.....	....
07_FU	C (1)	AY914102	–	–	0.10	....	....	.......G..	....
08_GC	D (1)	AY914103	–	–	0.10	....	....	T.........	....
11_LG	E (1)	AY914106	–	–	0.10	....	....A.....	T..G......	....
16_AZ	F (1)	AY914108	–	0.07	–	....	....AAATA.	....	....
20_AZ	G (1)	AY914112	–	0.07	–	..T....A.G	TG........	......G...	....
21_AZ	H (1)	AY914113	–	0.07	–	....	..C.......	....	....
24_AZ	O (3)	AY914116	0.02	0.14	–	CC........	....	....	....
29_AZ	P (1)	AY914120	–	0.07	–	.........G.	....	....	....
30_AZ	Q (1)	AY914121	–	0.07	–	......C...	...T......	C	.....C..
17_AZ	V (3)	AY914109	–	0.21	–	....	....	..T.......	....
18_AZ	W (1)	AY914110	–	0.07	–	....	.........T	..T..T....	....
24705_CH	I (2)	AY914137	0.05	–	–	....	....	....	..A.....
24707_CH	J (2)	AY914139	0.05	–	–	...T......	....	....	....
24717_CH	K (1)	AY914143	0.02	–	–	....	....	....	C.......
24727_CH	L (1)	AY914150	0.02	–	–	....	....	....	.......A
24729_CH	M (1)	AY914151	0.02	–	–	....	....	.G........	...TA.A.
24742_CH	N (1)	AY914161	0.02	–	–	....	.........T	........A.	....
35_MD	R (1)	AY914128	0.02	–	–	C.........	....	....	....
36_MD	S (1)	AY914129	0.02	–	–	....AT....	....	....	....
49_MD	U (1)	AY914136	0.02	–	–	....	....	....	.C......

TF: Tenerife, HI: El Hierro, FU: Fuerteventura, GC: Gran Canaria, LG: La Gomera, CH: Ilhéu Chão, MD: Madeira, AZ: Pico/Azores, CI: Canary Islands

Phylogenetic analysis

As genetic distances were rather small, no tree-building algorithm revealed any conclusive phylogenetic or phylogeographic patterns. Variable characters and haplotypes in the data set are documented in Table 14. The minimum spanning network showed a clearly starlike topology connecting all 22 haplotypes (Figure 20). In the centre, the most common sequence haplotype (haplotype A) is shared by 37 individuals from eight different islands including all three major geographic regions. The remaining haplotypes can be derived from this sequence through only 1–6 nucleotide substitutions. Almost all of the latter haplotypes are confined to just one island or geographical region and are not shared between the island groups. The only exception is haplotype O which was found in two individuals on Madeira and once on the Azores (Figure 20, hatched circle). This suggests a

recent range expansion which is also confirmed by significantly negative values of Tajima's D in all populations (Table 12).

From the pairwise mismatch distribution no population structuring can be detected (Figure 21). The observed frequencies formed a curve very similar to the expected shape of the curve after the model of sudden expansion and respective values are significantly correlated ($P < 0.01$).

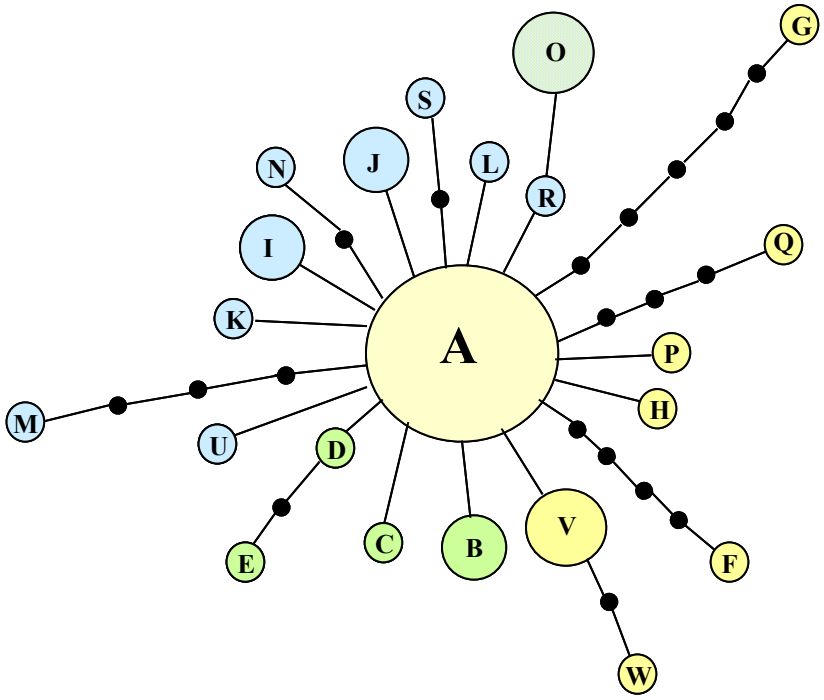

Figure 20 Minimum spanning network for 22 haplotypes (A-S, U-W, see Table 14 for reference) of 65 samples from *Serinus canaria* in Macaronesia based on 1 000 bp of the mitochondrial cytochrome *b*-gene
Haplotype frequency is indicated by dot size and origin of haplotypes is described by different colours (yellow: Azores; blue: Madeira, including Ilhéu Chão; green: Canary Islands). Only haplotypes **A** and **O** are shared between different archipelagos.

Morphological data

Morphological data of a total of 261 birds from Pico/Azores, Ilheu Chão and Madeira were analysed and revealed significant differences between these populations in all three measurements (Figure 22).

Wing length did not show a consistent pattern across populations ($F_{2,253} = 6.35$, $P = 0.002$). Generally, females had shorter wings than males ($F_{1,253} = 53.93$, $P < 0.0001$). Between populations, the only significant result was that birds from Madeira had shorter wings than those from Ilheu Chão ($P = 0.008$).

Beak size showed significant differences across populations ($F_{2,253} = 17.46$, $P < 0.0001$) and also a sexual dimorphism ($F_{1,253} = 13.80$, $P < 0.0002$). For example, beak size was

smaller in the individuals of Pico compared to those of Ilheu Chão ($P < 0.0001$) and Madeira ($P = 0.014$).

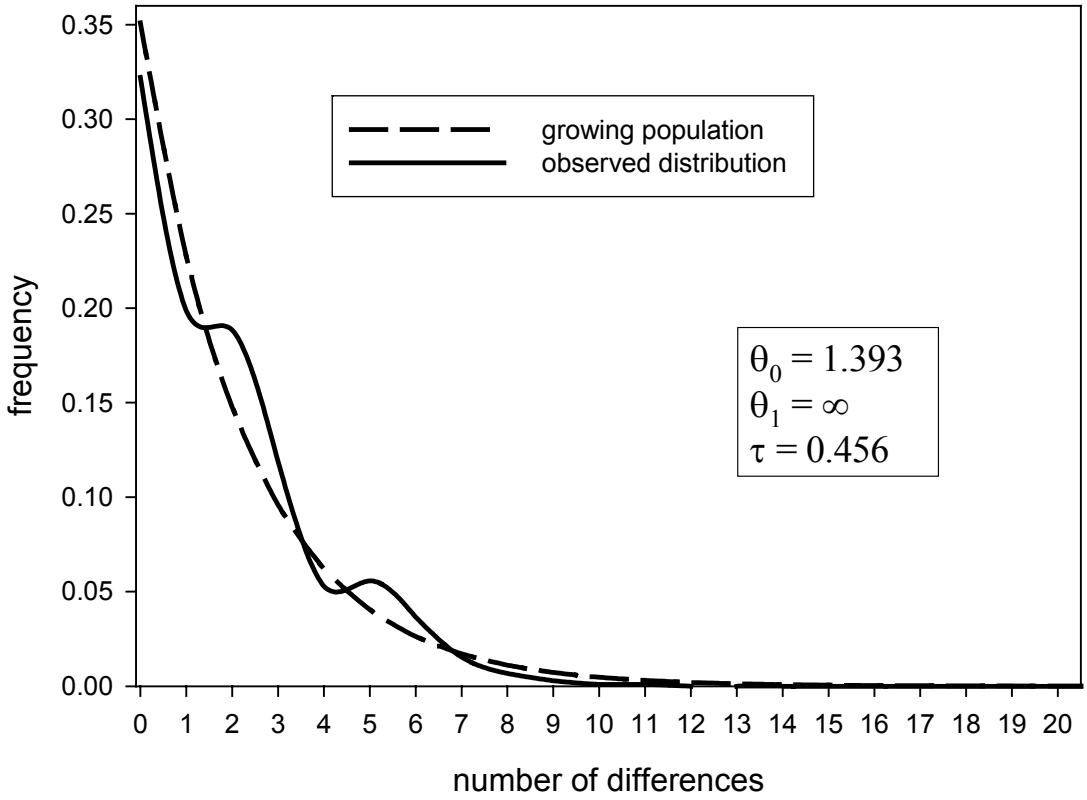

Figure 21 Pairwise mismatch distribution of 65 samples from *Serinus canaria* mitochondrial cytochrome b sequences in Macaronesia
The observed frequencies were very similar to the expected values, revealing no population structuring. $\theta = 4N\mu$ with N describing the effective population size and μ the mutation rate per gene. $\tau = 2\mu t$ describes the date of population growth in units of mutational time.

Body weight significantly differed across populations ($F_{2,255} = 33.43$, $P < 0.0001$) but did not show sex differences ($F_{1,255} = 0.13$, $P = 0.721$). This measure was largest in the Pico population, followed by Ilheu Chão and Madeira populations ($P < 0.0001$ for all comparisons).

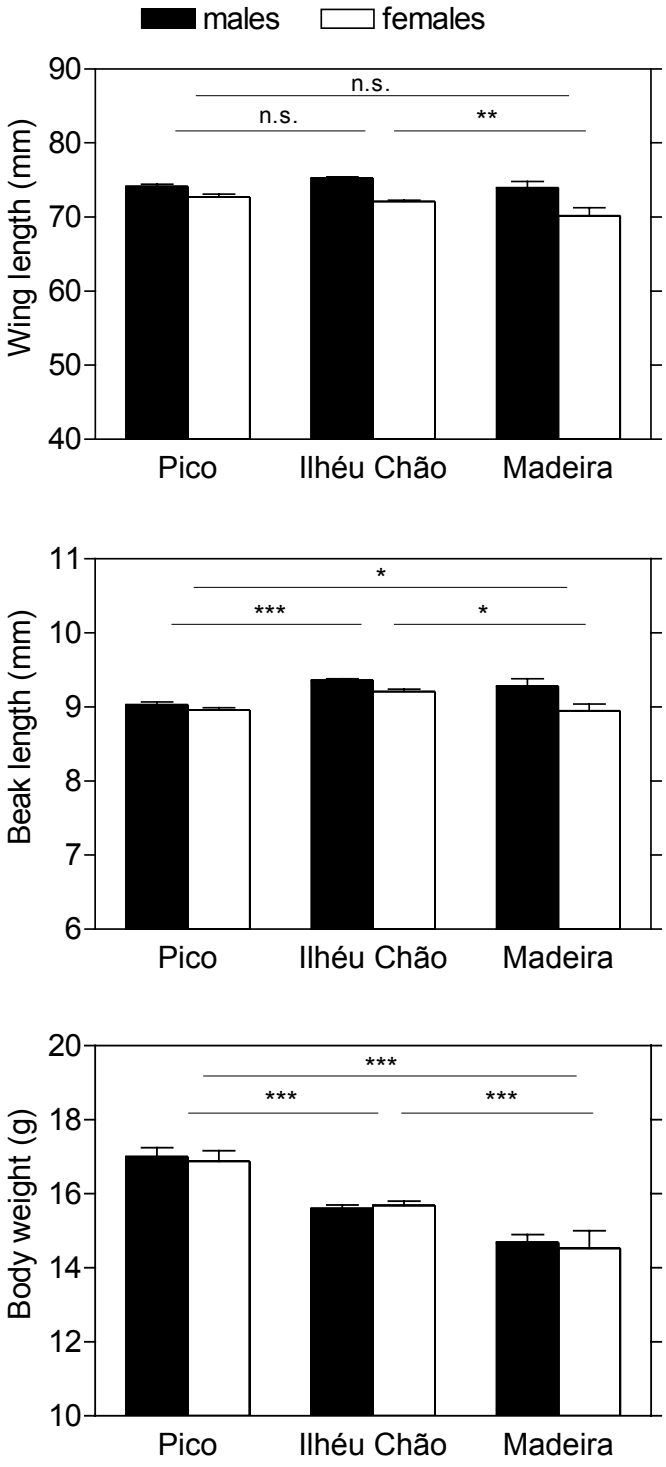

Figure 22 Morphological measurements of the individuals on Pico (Azores) (n = 28), Ilhéu Chão (n = 205) and Madeira (Madeiran archipelago) (n = 28), split by males and females
Lines upon bars indicate the significance level of inter-population comparisons. Values are means ± SE.

3.2.4 Discussion

The surprising result that genetic distances within and between geographically distant island canary populations, based on mt cytochrome b sequences were very low and ranged mainly between 0.1 and 1 % with means of up to 0.45 % (Table 13) could indicate:

The colonisation of the Atlantic islands by island canaries did occur rather recently. A sudden range expansion is also corroborated by the demographic history (Figure 21), Tajima's D values and the topology of the minimum spanning network (Figure 20). Accepting 0.4 % of sequence divergence per million years as was estimated for the genus *Serinus* before (Arnaiz-Villena et al. 1999), the diversification within *S. canaria* occurred around 1.1 million years ago. With the same rate the data suggest the divergence of *S. serinus* and *S. canaria* had occurred around 4.3 million years ago, while Arnaiz-Villena et al. (1999) date this event at 3.5–4.0 million years ago. The same authors present evidence for a rapid radiation of the whole genus *Serinus* with only up to 4 % average nucleotide divergence between distantly related species, a low within-species variability (< 0.3 %), and incomplete reproductive barriers between distantly related species.

An alternative explanation that would cause small genetic distances is an ongoing substantial gene flow between the different island groups leading to genetic uniformity. The minimum spanning network shows a clearly star-like phylogeny and the geographical confinement of most of the haplotypes to certain islands or island groups (private haplotypes) contradicts substantial gene flow. Furthermore, the N_m value well below 1.0 does not support high gene flow either. But it has to be considered that the sample size for some islands was rather small which could influence the detected haplotype distribution. However, the phylogenetic analysis convincingly shows weak island-specific differences between all three archipelagos investigated. It is likely that due to the usually rather slow mutation rate of the mitochondrial cytochrome b gene and the involved relatively short time span the genetic differentiation is underestimated here. A faster evolving genetic marker system (e.g. mitochondrial control region or microsatellites) could confirm these first hints towards a slight genetic differentiation of island canaries in Macaronesia.

All the Macaronesian archipelagos are of volcanic origin and some islands are much younger than others, thus covering a broad range of geological ages. For example, Pico (Azores) is only about 200 000 years old, whereas the origin of Madeira and some of the Canary Islands was up to 20 million years ago. This was well before the divergence of the closely related species *S. serinus* and *S. canaria* that occurred about 3.8 million years ago in the Mediterranean region (Arnaiz-Villena et al. 1999). Generally, it is assumed that the colonisation of the Azores by island canaries originated from Madeira (Bannermann & Bannermann 1966), which, according to the data presented here, could have occurred quite recently (650 000–725 000 years ago). The one haplotype shared in birds from these two archipelagos supports this hypothesis. In conclusion, the Canary Islands were also colonised from Madeira around 375 000 years ago. Despite a considerable distance

between the different archipelagos, genetic distances have not diverged to a larger scale. This pattern is similar to genetic data from the Sardinian warbler (*Sylvia melanocephala*) in the Canary islands and the Mediterranean region which shows almost no genetic diversification across their range (chapter 3.5). The finding of small genetic distances (median 0.1 %) and the weak phylogenetic differentiation in the Island Canary are in contrast to recent studies on other passerines in Macaronesia (e.g. the chaffinch *Fringilla coelebs*, European robin *Erithacus rubecula*, blue tits *Parus caeruleus*, goldcrests *Regulus* spp.) which revealed a strong substructuring with highly distinct taxa even on neighbouring islands (Marshall & Baker 1999, Dietzen et al. 2003, Kvist et al. 2005, Päckert et al. 2006).

At first sight, a substantial gene flow between islands or even between the three archipelagos in the Island Canary seems unlikely as it represents a rather sedentary species and individuals stay in their breeding areas all year round. This fact has been confirmed in a population on a small island of the Madeiran archipelago (Voigt & Leitner 1998). On the other hand, there are also reports of larger scale movements. Bannermann & Bannermann (1966) anecdotally reported that canaries disappeared for several months from the Azorean island of Terceira. Furthermore, there are reports that the canaries migrate from the island of Flores and stay during winter on the island of Corvo which is 24 km distant (Knecht & Scheer 1971). These observations indicate at least a potential ability of canaries to travel and disperse over larger distances. One also has to consider the strong prevalent North Atlantic winds, including the trade winds that regularly lead to considerable long-distance drifts of migrating birds and consequently to the colonisation of distant archipelagos.

There is also a possibility of repeated colonisation events and back-colonisations as was proposed for the chaffinch and robin populations on the Canary Islands (Marshall & Baker 1999, Dietzen et al. 2003). Concerning the mating system, island canaries are socially monogamous, as recently no evidence was found for EPP in the population of the only 0.5-km2 island, Ilheu Chão (Voigt et al. 2003). At the nearest point, Ilheu Chão and Madeira are 20 km apart, forming only a small barrier against possible gene flow. In consequence, no island specific genetic pattern exists between these populations. In a study on house sparrows (*Passer domesticus*), Griffith et al. (1999) also found a similar genetic pattern in three different populations, including one on a very small (3 km^2) island. Surprisingly, the authors found different levels of EPP between the three populations. They concluded that their island population is not effectively isolated because it is only 20 km from the mainland, comparably to the situation on the Madeiran archipelago, where genetic distances remain very small.

Rather small genetic distances also occur in populations of Galapagos ground and tree finches. In contrast to the canaries, these finches have been classified into different sympatric species within the genera *Geospiza* and *Camarhynchus* on the basis of

morphological differences (Gould 1837, Grant 1986, Sato et al. 1999). This led to the assumption that speciation can occur within low genetic distance values. However, Sato et al. (1999) also point out that the morphological traits in ground and tree finches are not necessarily reliable characters to distinguish the different species; moreover, the variation of each of the body characteristics are often overlapping between species. Together with the molecular data that also failed to reliably distinguish between these species, Sato et al. (1999) conclude that the individuals of different morphologically identified taxa represent an intermixture rather than clearly defined species as had been assumed previously. A comparable, but less concise, scenario can be found in this island canary study. Here, the intra- and inter-population genetic distances overlap and a distinct phylogeographic pattern cannot be recovered.

However, in these populations, subtle yet recognisable morphological differences between islands were obvious. Beak size and body weight differed significantly between the individuals of Pico and the other islands. Wing length showed some differences, although no consistent pattern occurred. Emerging differences in beak size could well reflect different dietary requirements of the birds. Although there are similarities in the plant communities within Macaronesia, there are distinct particularities across archipelagos or even islands, which is partly reflected in a variety of endemic species (Press & Short 1994, Sjögren 2001). Anyway, the subtle morphological differences in the canaries do not correspond to the genetic profile in this study. In conclusion, it can be stressed that there are clear indications for a weak phylogeographic and morphological differentiation within Macaronesia, but due to the recent origin of the island populations the mitochondrial cytochrome b gene does not yet reveal a clear intraspecific differentiation and thus prevents a sound conclusion on phylogeography, colonisation and radiation by the island canary.

The genetic variation of a population may reflect a variety of factors like demographic effects, leading to population bottlenecks, mating systems, leading to different effective population sizes, and dispersal and migration, leading to gene flow. The intensity of sexual selection may be lower in island populations, emerging from alternative strategies like long-term pair bonds and assortative mating. The morphological differences found in this study are certainly due to natural selection processes that may be able to change the size and the shape of birds on different island environments in a faster way than evolving cytochrome b sequences. The question to what extent sexual selection plays a role in promoting morphological or behavioural differences could be investigated by looking at behaviours related to reproduction. For example, the song of songbirds is involved in territorial defence and mate attraction (Catchpole & Slater 1995). In canaries of a Madeiran population, it is now well documented which parts of the song are likely to be involved in mate choice (Leitner et al. 2001). The song differentiation of birds living in the

Macaronesian region has already been studied in detail in a number of species, such as the chaffinch (Lynch & Baker 1994) and the crests and kinglets (Päckert et al. 2003, Päckert et al. 2006). Investigating and comparing the song characteristics of Island Canary populations and a molecular marker system with faster evolutionary rates are certainly the next steps to further understand their phylogeny and phylogeography.

3.3 Radiation of Atlantic goldcrests (*Regulus* spp.) [ii]

3.3.1 Introduction

Fauna and flora of the Canary Islands mainly embrace elements from the palearctic region and developed a relatively high degree of endemism despite its geographic proximity to mainland Africa. Of 365 vertebrate species on the Canarian Archipelago 5.75 % are endemics including some endangered bird species like the Teyde Finch (*Fringilla teydea*) and the laurel pigeons, *Columba bolli* and *C. junionae* Garcia Casanova & Rodriguez Luengo 1998). Thirteen bird species of the Canarian avifauna including the Canary (*Serinus canaria*) are also present on other Atlantic Islands, the Azores and Madeira, most of them showing inter-insular subspeciation (species list in Knecht & Scheer 1971). In several of the local passerine taxa, recent molecular and bioacoustic research has revealed high degrees of differentiation between populations from the Canary Islands and their geographic counterparts from mainland Europe. Accordingly, species rank was assigned to several avian subspecies, the Tenerife Goldcrest *Regulus teneriffae* (Sibley & Monroe 1990), the Canary Islands Chiffchaff *Phylloscopus canariensis* (Helbig et al. 1996), the Ultramarine Tit *Parus teneriffae* (Salzburger et al. 2002a) and the Canarian Robin *Erithacus superbus* (Bergmann & Schottler 2001). In addition, Dietzen et al. 2003) discovered a strong substructuring within Canarian Robins, namely between Gran Canarian and Tenerifean populations opposing those of the other islands and Europe. High genetic and acoustic differentiation was also reported in the Madeiran Firecrest, *Regulus madeirensis* (Päckert et al. 2001, Päckert et al. 2003).

Close relatives of the two European regulid species, Goldcrest (*Regulus regulus*) and Firecrest (*Regulus ignicapillus*), are present on all Macaronesian islands except for the Cap Verde Islands. Apart from the Firecrests on Madeira, Goldcrests inhabit the relict laurisilva forests and mountain vegetation on the Azores and the Canary Islands. On six Azorean islands the Goldcrests are represented by three subspecies (*Regulus regulus azoricus, R. r. sanctaemariae* and *R. r. inermis*). These populations are considered recent descendants of mainland Goldcrests *R. r. regulus* (Volsoe 1951), which has been confirmed by cytochrome b sequence analysis (Päckert et al. 2003). However, phylogenetic relationships among the Azorean subspecies cannot be resolved by cytochrome b data. Distribution patterns of song types revealed two major dialect groups within the Azorean goldcrests, i.e. one on the eastern islands São Miguel and Santa Maria vs. another one including all populations on the central and the western islands group (Päckert & Martens 2004).

[ii] The mitochondrial control region was sequenced (L. Kvist and co-workers) and analysed (M. Päckert and co-workers) within a collaboration of several institutes. Bioacoustic analyses were conducted by M. Päckert and co-workers. Because they are a vital component for the interpretation of results, they are included here, although only sequences and subsequent analysis of mitochondrial cytochrome b data and morphological measurements of live birds are derived by myself.

The discussion on the taxonomic status of the Tenerife Goldcrest (*Regulus r. teneriffae*), apart from its status as a subspecies or an independent species, has been controversial ever since. For morphological and zoogeographic reasons it was regarded as closely related or even conspecific with the Firecrest (Volsoe 1951, Vaurie 1954, Voous 1962), but also a close relation to the Goldcrest was taken into account (Hartert 1907, Bannermann 1922). The latter hypothesis is corroborated by bioacoustic analyses (Becker 1978, Martens et al. 1998). The ethological repertoire of *R. r. teneriffae* comprises behavioural components similar to those of European *R. r. regulus*, but also some primitive features for which it was considered an ancestral regulid taxon of valid species rank (Löhrl & Thaler 1980). Since DNA-hybridization data by Sibley & Monroe (1990), and 16S rNA sequence data by Sturmbauer et al. (1997) confirmed its genetic differentiation against European *R. r. regulus*, the Tenerife Goldcrest was upgraded as a sixth valid regulid species, *R. teneriffae* by many authors. A more comprehensive intrageneric cytochrome b phylogeny of the regulids showed that all western Palearctic goldcrests (Azores, Canary Islands and nominate *R. r. regulus* from Europe) are of monophyletic origin, and are only distantly related to the Asian goldcrest subspecies *R. r. tristis*, *R. r. japonensis* and *R. r. himalayensis* (Martens & Päckert 2003, Päckert et al. 2003). Conclusively, the Tenerife Goldcrest is treated as a subspecies, *R. r. teneriffae*, within the entire goldcrest assemblage (Martin & Lorenzo 2001, Dickinson 2003).

The Tenerife Goldcrests inhabit the western islands Tenerife, La Gomera, El Hierro and La Palma, while they are missing on the easternmost islands Gran Canaria, Lanzarote and Fuerteventura (Martin & Lorenzo 2001). Genetic and bioacoustic studies on inter-insular differentiation in goldcrests on the Canary Islands are lacking so far. Subspecific differentiation within the Canarian archipelago has never been considered.

This molecular study intends to clarify the interinsular relationships of the Canarian and the Azorean goldcrests. Samples were included from all Canarian populations and from nearly all Azorean islands inhabited by goldcrests. For phylogenetic reconstructions two working groups independently sequenced a 782 bp fragment of the mitochondrial control region (external data by M. Päckert and L. Kvist) and a 1 351 bp fragment of the mitochondrial ND5/cytochrome b gene (own results). The genetic distances calculated from control region and cytochrome b sequences are compared and provide estimates of absolute substitution rates based on paleogeographic data for both genes.

3.3.2 Material and Methods

A total of 48 DNA samples (control region) and 51 DNA samples (ND5/cyt b) of western palearctic goldcrests from the Atlantic Islands (Canary Islands: *R. r. teneriffae*; Azores: *R. r. azoricus, R. r. sanctaemariae, R. r. inermis*, Madeira: *R. madeirensis*) and nominate *regulus* and *R. ignicapillus* from western and Central Europe were studied. As outgroups four samples of Asian goldcrests (*R. r. tristis, R. r. yunnanensis* and *R. r. japonensis*),

which turned out to be only distantly related to western palearctic goldcrests (Päckert et al. 2003), and one sample each of the Golden-crowned Kinglet *Regulus satrapa* and the Ruby-crowned Kinglet *Regulus calendula* were used. Origins of samples are given in Appendix A.

About 780 bp of the mitochondrial control region (first and parts of the second domain) were amplified using primers RegND6midL (5'-AAT AAT CCC CGC CAC AAT AA-3'), and Reg12SrRNAH (5'-AAC AGT AAG GTT AGG ACT AA- 3'). The PCR protocol was 94°C for 2 min followed by 30 cycles of 94°C for 45 s, 50°C for 30 s and 72°C for 2 min with a final extension in 72°C for 5 min. Sequencing of the PCR products was performed with BigDye™ v. 3.0 and v. 3.1 Dye Terminator Cycle Sequencing Kits (Applied Biosystems) according to the manufacture using primer Reg370L (5'-CTA GTG TAC GAG GAA TGT C-3') or STH411 (5'-AAA TAA CCA GGT TCT CTG GCT TG-3') and reactions were electrophoresed with the ABI 377 automatic sequencer.

Furthermore 1 351 bp of the mitochondrial ND5 and cytochrome b genes were amplified by PCR as described in Dietzen et al. (2003) using the specific primers L14464 (5'-CTW GGC AGC ATT AYA GCA GG- 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'). Before 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 L14464, 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 with 10 s at 96°C, 5 s at 52°C and 4 min. at 60°C. Products were purified and sequenced as described by Dietzen et al. (2003).

The sequences were aligned by eye using the BioEdit program (Hall 1999). Both data sets yielded similar results. Nucleotide diversity, π (Nei 1987), haplotype diversity, $\hat{h}$ (Nei 1987), $\theta = 2Nf_e\mu$ (Tajima 1996) and mismatch distributions with the raggedness index (Harpending 1994) were estimated with DnaSP v. 3.51 (Rozas & Rozas 1999). Analysis of molecular variance using Tamura-Nei distances and haplotype frequencies, and the pairwise Φ_{ST} -values were calculated with Arlequin v. 2.0 (Excoffier et al. 1992). For the molecular variance analysis the sequences were grouped in two levels: the first level according to larger geographic regions (Europe, Azores, Canary Islands, Asia) and the second level mainly according to the subspecies represented by the samples. A single sample representing subspecies *sanctaemariae* was included into the sample set of *azoricus* and the samples from the Canary Islands were divided into two groups, one group including samples from El Hierro and La Palma, and the other one including samples from

Tenerife and La Gomera. For estimates of past gene flow and possible colonization routes of goldcrests between continental Europe and the Atlantic islands asymmetric migration rates were calculated between the main phylogeographic clusters with migrate v.1.7.3 (Beerli 1997-2003). Effective population size of females was estimated from theta values ($Nf_e = \theta/2\mu$), and following Avise et al. (1988): $Nf_e = 0.5 \times 10^8$ p/g, where p is the mean intraspecific genetic distance and g the generation time (long term effective population size). Generation times of great and willow tits (*Parus major, P. ater*) were calculated from lifetime tables by Kvist et al. (1999): 1.97 and 2.26 years. Referring to these values, an approximate value of g = 2 years was used.

An appropriate substitution model for the molecular data set was estimated via Likelihood Ratio Tests with Modeltest 3.04. (Posada & Crandall 1998). The Asian samples (control region) and *R. ignicapillus/R. madeirensis* (cytochrome b) were included into the data set. The selected model for the control region was the Tamura-Nei model, TrN+I (Tamura & Nei 1993) for all *Regulus* haplotypes. Likelihood settings were as follows: empirical base frequencies: pA = 0.226, pC = 0.307, pG = 0.174, pT = 0.293; proportion of invariable sites I = 0.9028; substitution rates: R = 1, except $R_{[A-G]} = 20.4$, $R_{[C-T]} = 4.9$. For the cytochrome b data set a modification of the Hasegawa-Kishino-Yano (HKY85) model, TVM+I+G (Posada & Crandall 1998) was most appropriate with empirical base frequencies pA = 0.3032, pC = 0.3233, pG = 0.1262, pT = 0.2473; I = 0.5430, Gamma distribution shape parameter $\alpha = 0.7623$; $R_{[A-C]} = 2.8772$, $R_{[A-G]} = 17.9235$, $R_{[A-T]} = 2.5034$, $R_{[C-G]} = 0.6404$, $R_{[C-T]} = 17.9235$ and $R_{[G-T]} = 1.000$.

Phylogenetic trees were constructed under different approaches: Neighbor Joining and Maximum Parsimony (NJ, MP; Saitou & Nei 1987), PAUP 4.0.1 (Swofford 2001), Maximum Likelihood (ML, TreePuzzle, Schmidt et al. 2000) and Bayesian inference of phylogeny (Huelsenbeck & Ronquist 2001). Robustness of clades was estimated by 1 000 bootstrap replicates (Felsenstein 1985), via quartet puzzling in ML, 1 000 puzzling steps (Strimmer & von Haeseler 1996) and via Bayesian posterior probabilities using Markov chain Monte Carlo (MCMC, 500 000 generations, burnin = 3 000). To verify the molecular clock hypothesis, a constant rate test was carried out with Tree Puzzle (Schmidt et al. 2000). Genetic distances were calculated using the likelihood settings of the selected model. Amongst others, SH test (Shimodaira & Hasegawa 1999), two-sided KH test (Kishono & Hasegawa 1989) were applied to different tree topologies using TreePuzzle.

For estimating absolute rates of molecular evolution (r_{loc}) for the control region and cytochrome b and divergence times between *Regulus* lineages r8s 1.70 (Sanderson 2004) was used. The input files included the haplotype NJ trees (cyt b and cr) with each haplotype represented once. In order to cover a maximum of genetic variation, especially in the Azorean subspecies group, the cyt b data set was increased by the sequences of 27 further haplotypes from a former study (Päckert et al. 2003). For NJ reconstruction

sequences were cut down to 585 bp. Alternative runs were performed with reduced input trees in which each taxon was represented by a single, the most common haplotype. Zero branch lengths were automatically collapsed and turned into hard polytomies (‘‘r8s 1.70’’, command: ‘‘collapse’’). For reconstruction of divergence times and absolute rates of substitution nonparametric rate smoothing was applied, NPRS (Sanderson 2004) with POWELL algorithm. Some nodes of the molecular phylogeny were constrained to paleogeographic data, which should roughly correspond to lineage splits in crests and kinglets, i.e. age estimates of volcanic islands. This paleogeographic approach was applied to cyt b data of Hawaiian honeycreepers of the subfamiliy Drepanidinae and yielded a substitution rate of 1.6 % per my (Fleischer et al. 1998). Paleogeographic data are available for volcanic Atlantic islands of the Azorean and Canarian archipelagoes, too. As time estimates for most of these paleogeographic events comprise time spans like oldest and youngest lava shields rather than a fixed point in time, nodes of input trees were constrained to a minimum and a maximum age.

Five paleogeographic events that gave rise to geographic barriers or new habitats, for instance on volcanic islands, were used as calibration points. 1) The most recent split between Nearctic and Palearctic faunal elements (*R. regulus* vs. N American *R. satrapa*) is supposed to have originated from the last opening of the Bering Strait which was estimated by mollusc and diatomean fossil records at 4.7-7.4 my ago (Marincovich & Gladenkov 1999). 2) The beginning of the Pleistocene is traditionally referred to an increase of cryophilic nanoplankton at 1.7 my ago (Aguirre & Pasini 1985), but first cold periods and continental ice shields arose earlier and lead to a first decline of tertiary forests about 2.4 my ago (West 1988). The upcoming fragmentation of the continuous coniferous Taiga belt is considered as the cause for the initial separation between ancestors of east palearctic (early stock of recent *R. r. tristis* and *R. r. japonensis*) and west palearctic goldcrest populations (recent *R. r. regulus* and populations of the Atlantic islands). 3) The arrival of ancestors of *R. r. teneriffae* on the Canary Islands is referred to the phases of volcanic eruptions, which gave rise to the island of Tenerife. The oldest massifs of the Tenerife complex, the Teno massif in the south (12-4.5 my) and the Anaga mountains in the east (6.5-3.5 my), were not connected until a final eruptive phase which built up the Canadas and the Dorsal Ridge 1.9-0.7 my ago (Ancochea et al. 1990). As calibration points for first *Regulus* settlement on Tenerife the time span of low volcanic activity on the Tenerife complex between the end of the first and the beginning of the last eruptive phase between 4 and 1.9 my is used. The western islands La Palma and El Hierro are considerably younger (1.7-1.1 my). 4) The age constraint for the first invasion of *R. regulus* to the Azores refers to paleogeographic age estimates by Johnson et al. (1998) between the uprise of the oldest and the youngest parts of São Miguel (eastern population, Furnas: 0.7 my ago; western population of Sete Cidades: 0.2 my ago), and finally, 5) The islands of the central and the

western group inhabited by recent *R. r. inermis* are considerably younger (Pico: 0.37-0.3 my; Chovellon 1982). To morphologically verify possible genetic splits between Canarian populations the rich Canarian skin series of Museum Alexander Koenig, Bonn, was studied. Of 36 specimens (Tenerife: n = 10, La Gomera: n = 12, La Palma: n = 8, El Hierro: n = 6) measurements were taken: wing length (maximum chord), tail length (emergence point of central rectrices from skin to tip), bill length (from skull to tip) and tarsus length. Checked museum skins were collected by Thanner within a short period in winter and the pre-breeding season in 1904 and 1905. They are in excellent condition and well suitable for colour comparison. Further morphological measurements were taken from live birds between spring 2002 and autumn 2003 (La Palma: n = 6, El Hierro: n = 2, La Gomera: n = 10, Tenerife: n = 12, Azores: n = 7, Madeira: n = 16): body mass, wing length, length of primaries P1 (innermost) to P9 (outermost), secondary S1, tarsus, bill width, bill length, bill height and distance from nostril to bill tip (NaLoSpi). Sliding calipers were used to get bill and tarsus length, calipers for tail length and a ruler for wing length. Also features of plumage coloration and head stripe pattern were taken into account.

For the bioacoustic analyses recordings of territorial song from Canarian goldcrests to trace possible differences of inter-insular song structures were used. The analysis of song is purely descriptive using sonagrams of territorial songs of 77 males (recordings by M.P., April 2003: El Hierro n = 15, La Palma n = 22, Tenerife n = 31, La Gomera n = 9). For all Canarian samples included in the molecular analysis of the mitochondrial control region a song recording of the corresponding male is available.

3.3.3 Results

Phylogeography

The 50 goldcrest sequences of the control region (47 Atlantic Islands, 3 E Asia) produced a 782 bp long alignment. There were 31 variable sites (4.0 %) in the alignment of which 19 (2.4 %) were parsimony informative and two one bp indels, while in the ND5/cyt-b alignment (1 354 bp), 64 sites (4.7 %) were variable and 54 (4.0 %) parsimony informative. These comprised 21 (cr) and 16 (cyt b) different haplotypes (haplotype diversity was 0.902 and 0.893, respectively). The nucleotide and haplotype diversities were the highest within the Canary Islands (π = 0.00547 and 0.00992, $\hat{h}$ = 0.769 and 1.000), the highest theta values (θ = 0.00443 and 0.00967) were also obtained from the Canaries (for cr and cyt b, respectively). All the lowest diversity values for the cr and cyt b were estimated from the birds of El Hierro/La Palma and Tenerife/La Gomera, respectively. Diversity estimates are shown in Table 15.

Table 15 DNA polymorphism in the study populations
π= nucleotide diversity, $\hat{h}$= haplotype diversity, $\theta = 2Nf_e\mu$, r_{loc}= local substitution rate (substitutions per site per my; estimated with "r8s" for the according clade; ** = mean values of all terminal clades), Nf_e= effective population size of females; Canary Islands: TEN= Tenerife, LG= La Gomera, EH= El Hierro, LP= La Palma; *= significance on a $p < 0.05$ level.

a) control region [1]

Population	π	θ	$\hat{H}$	number of haplotypes	Tajima's D	raggedness	r_{loc}	Nf_e	N
Azores	0.00143	0.00246	0.619	7	-1.34	0.0695	0.0064	217700	22
R. r. inermis	0.00120	0.00193	0.450	5	-1.25	0.1506	0.0032**	127309	16
R. r. azoricus [2]	0.00128	0.00168	0.600	3	-1.23	0.0622*	0.0000**	203390	6
Canary Islands	0.00547	0.00443	0.769	6	0.9295	0.1049	0.0030	-	14
TEN and LG	0.00128	0.00168	0.600	3	-1.23	0.0622*	0.0017	394366	6
EH and LP	0.00064	0.00099	0.464	3	-1.31	0.1671	0.0040	97059	8
Europe, *R. r. regulus*	0.00153	0.00212	0.758	5	-1.02	0.1111	0.0052	224576	12

b) ND5/cyt *b*

Population	π	θ	$\hat{H}$	number of haplotypes	Tajima's D	raggedness	μ	Nf_e	N
Azores [3]	0.00118	0.00130	0.800	4	-0.46983	0.0756	0.0060	131579	6
Canary Islands	0.00992	0.00967	0.766	8	0.10091	0.0949	0.0081	-	22
TEN and LG	0.00057	0.00065	0.647	4	-0.32096	0.2033	0.0063	49618	18
EH and LP	0.00314	0.00313	1.000	4	0.03892	0.1111	0.0075	159580	4
Europe, *R. r. regulus*	0.00099	0.00130	0.800	4	-1.29503	0.0933	0.0068	172414	6

[1] control region data contributed by M. Päckert and co-workers
[2] = one sample of *R. r. sanctaemariae* included into the data set of the eastern Azores
[3] = one sample of *R. r. inermis* included in *R. r. azoricus*.

The analysis of the molecular variance for the control region and ND5/cyt b sequences partitioned 38.8 % and 41.4 % of the variation between the main geographic regions, 47.8 % and 55.2 % between the groups, 13.44 % and 3.4 % within the groups, respectively. The variation is partitioned almost identically also when those three Asian samples are included (38.46 %, 47.81 % and 13.73 % for cr). The overall Φ_{ST} was very high, 0.8656 (cr) and 0.96578 (cyt b). Φ_{SC}, which describes the variation among groups within regions, was 0.7803 (cr) and 0.94165 (cyt b). Finally, Φ_{CT} describing the variation of groups among regions was 0.3880 (cr) and 0.41357 (cyt b). The pairwise Φ_{ST} values between the groups were all high (range 0.76216-0.9291, $p < 0.05$ for all), except between the populations on the Azores ($\Phi_{ST} = 0.29290$ between *R. r. azoricus* and *R. r. inermis*). The mismatch distributions of the control region and ND5/cyt b genes from the Azores and Europe were unimodal, contrasting to the distribution obtained from the Canary Islands (Figure 23). Tajima's D values for the Azores and Europe were negative, but not significantly. For the Azores however, the raggedness index was significant, supporting a recent growth in

population size there. Probably also the European population has experienced a sudden growth in its history, but a longer span of time has elapsed since. Within the Canary Islands, the mismatch distribution indicates population structuring (Figure 23). When the samples from the Canaries are divided into two groups (La Palma with El Hierro and Tenerife with La Gomera) both groups show an unimodal mismatch distribution, the group containing Tenerife and La Gomera having a significant raggedness index.

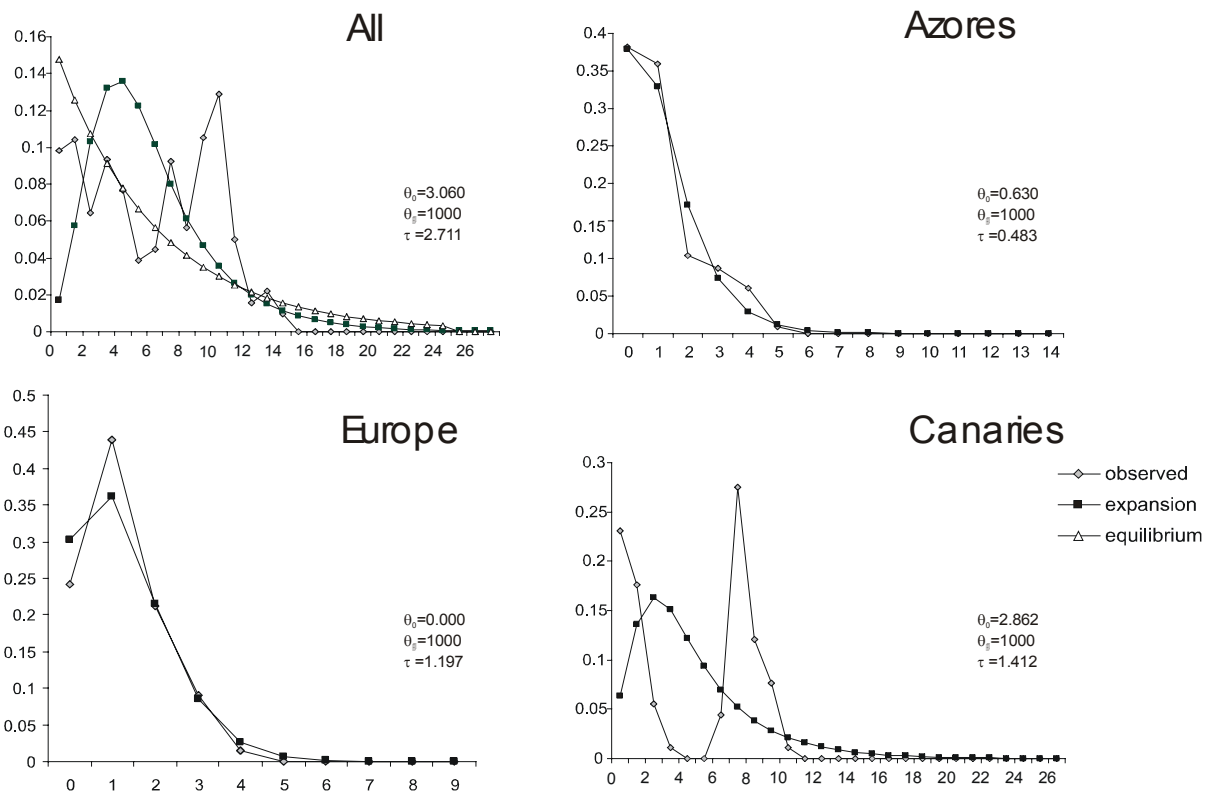

Figure 23 Mismatch distributions of samples from the geographical regions

All migration rate estimates are extremely low, in most cases less then one individual per generation. Even the highest value for migration from the Azores to the European continent accounts only 1.7 individuals per generation (asymmetric rate estimates are about 15 times higher than for the opposite direction from Europe to the Azores).

Molecular phylogenies for both data sets are shown in Figure 24 and Figure 25. In all phylogenetic reconstructions continental European goldcrests form a monophyletic cluster including all populations from the Azores, with highest support from Bayesian posterior probabilities. All haplotypes from Europe and from the Azores form a well-supported monophyletic cluster in the ML and Bayes trees (Figure 24 and Figure 25). In the NJ tree

(cr) haplotype C from southern Germany (Reg44) forms a sister clade to the poorly supported Azorean subspecies group (Figure 24).

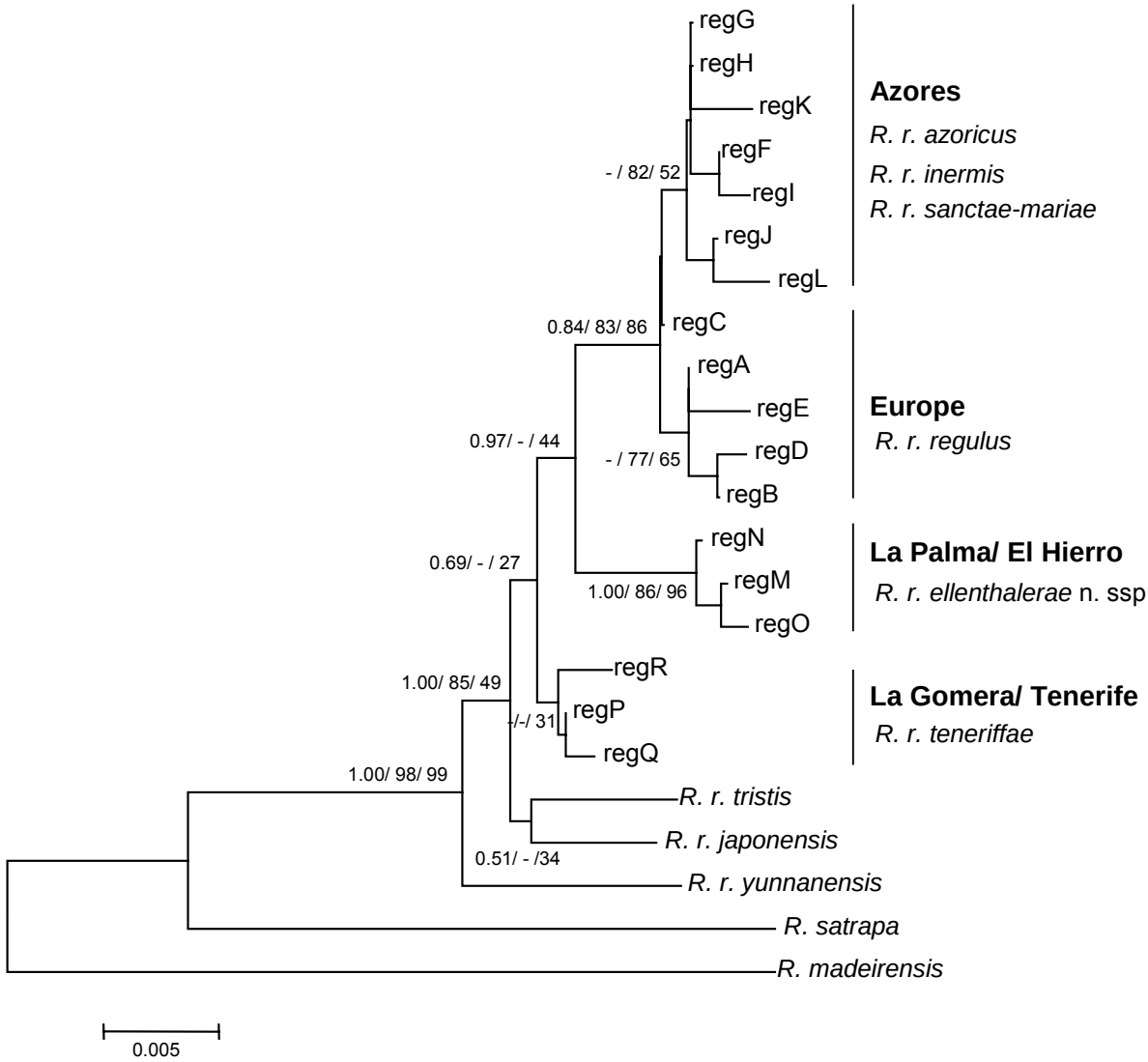

Figure 24 Neighbor Joining tree, 782 bp of the mt control region [1] for 17 haplotypes of western palearctic goldcrests

Clade support: Bayesian weights/ML/NJ (MCMC, 500 000 generations/1 000 puzzling steps/ 1 000 bootstrap replicates); the topology shown in the tree was best supported by SH and KH tests compared to six alternative tree topologies (p-SH= 1.00, P-1sKH= 1.00, log L= -1870.96).

[1] control region data contributed by M. Päckert and co-workers

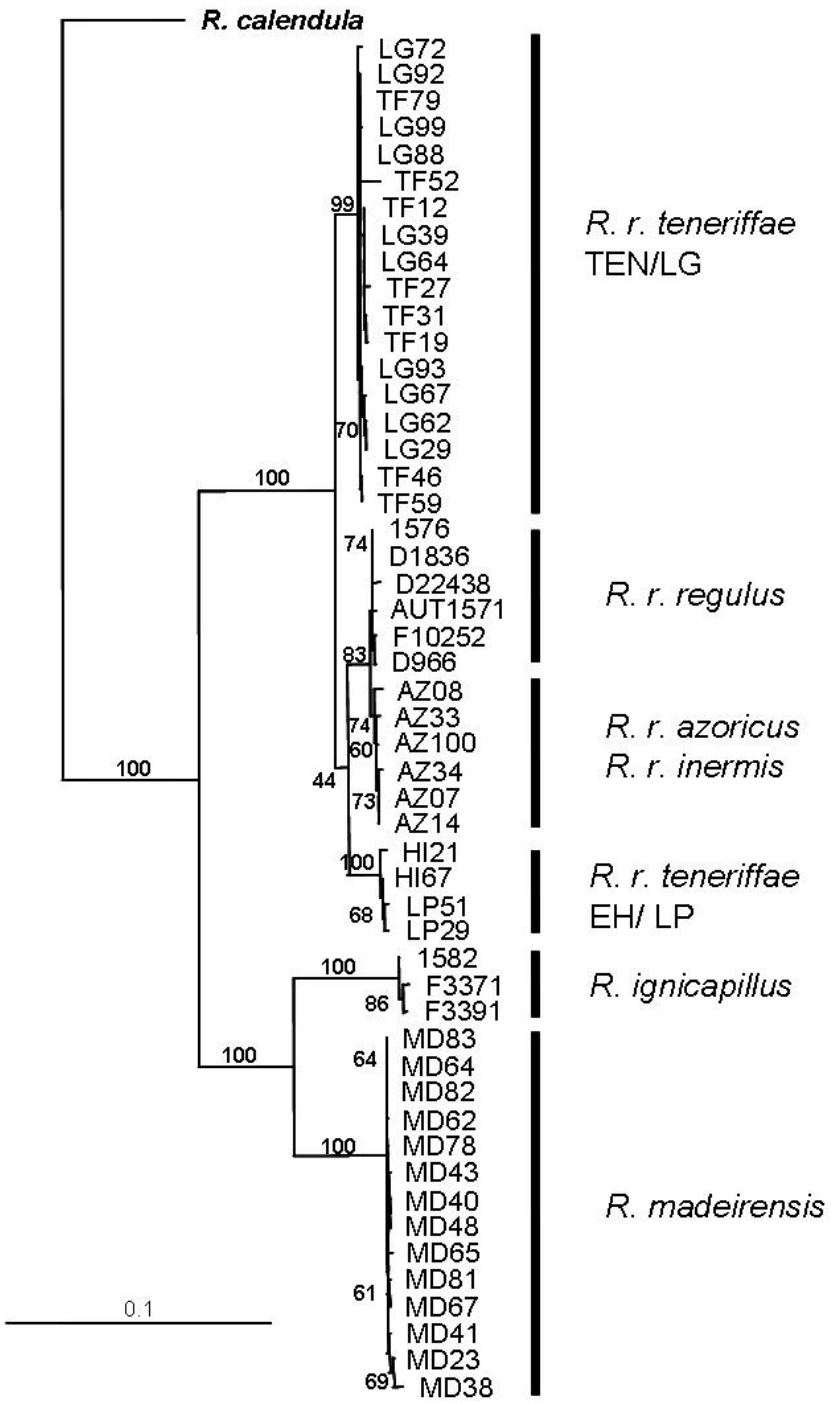

Figure 25 Maximum likelihood tree, 1 351 bp of the mt ND5/cytochrome b genes for 51 samples of *Regulus* sp.
Clade support: ML, 1 000 bootstrap replicates. TEN/LG = Tenerife/La Gomera, EH/LP = El Hierro/La Palma.

Two groups from the Canary Islands are deeply separated from each other: the northeastern group comprises haplotypes from Tenerife and La Gomera, and the

southwestern group includes haplotypes from El Hierro and La Palma. Monophyly of all Canarian haplotypes is not supported neither by cyt b, nor cr data. All haplotypes from southwestern islands El Hierro and La Palma belong to a well-supported monophyletic cluster in all reconstructions of cyt b and cr data. This southwestern Canarian cluster is a sister group to the European/ Azorean clade with highest support from ML and Bayesian analysis in the cr phylogeny and poor support in the cyt b tree (Figure 24 and Figure 25). Monophyly of the Tenerife/La Gomera group is well supported by cyt b data and poorly supported in the NJ reconstruction from the cr data set (Figure 24 and Figure 25). In ML and Bayesian reconstructions, haplotypes from the northeastern islands Tenerife and La Gomera form a basal polytomy with the main clade (not shown).

Among seven different tree topologies, the sister group relationship of the El Hierro/La Palma group and the European/Azorean assemblage including paraphyly of the *R. r. regulus* (RegC basal to the Azorean clade; Figure 24) was best supported by SH and one-sided KH tests (p-SH = 1.00, p-1sKH = 1.00, log L = -1870.96). However, the according phylogeny including a monophyletic European clade of nominate *regulus* yielded almost the same results (p-SH = 0.98, p-1sKH = 1.00, log L = -1870.96). Other phylogenetic hypotheses received poorer support from topology tests but were not significantly worse, i.e. monophyly of all Canarian haplotypes, monophyly of all Asian haplotypes (subspecies *japonensis*, *tristis* and *himalayensis*) and sister group relationship of the Tenerife/La Gomera group and the European/Azorean clade. However, only one topology was significantly excluded by SH and KH tests, namely a phylogeny suggesting monophyly of all Atlantic island haplotypes (Canary Islands and Azores as a monophyletic group: p-SH = 0.032, p-1sKH = 0.017, log L = -1889.91).

The minimum spanning tree for the European and the Atlantic goldcrest haplotypes (cyt b) is shown in Figure 26. Haplotypes from European and Azorean populations differ by 1-8 substitutions. The rare *R. r. regulus* haplotype *n* from southern France (F10252) and haplotype *k* from the Azores are separated by only two substitutions. Haplotype *k* was found in only one individual sample from São Miguel. All remaining Azorean haplotypes have derived from haplotype *k*, including the most common one (*i*) which was found in three individuals from São Miguel (Figure 26). Within the European clade three individuals from Germany share the most common haplotype *m*. The two clades from the Canary Island populations differ by 61-66 substitutions. The most common haplotypes of these two groups were found in one and ten samples, respectively.

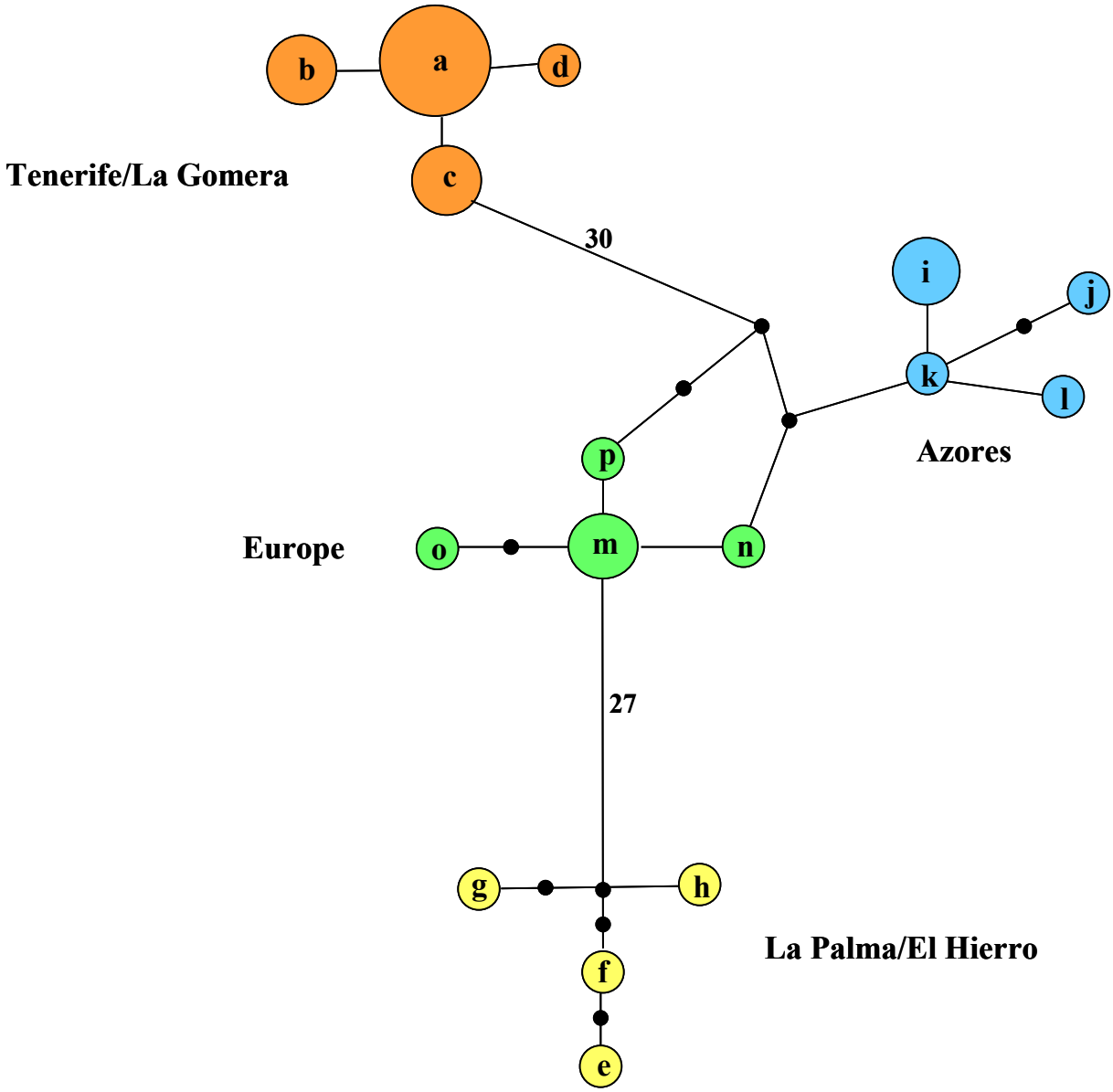

Figure 26 Minimum spanning tree for 16 haplotypes (given in lower case letters a-p) of 34 western palearctic goldcrest samples
1 351 bp of the ND5/cyt b genes. Size of circles indicates frequency of haplotypes. Black dots equal one nucleotide substitution. Numbers along branches give number of nucleotide substitutions between two haplotypes.

According to mitochondrial DNA sequences, genetic distances (TrN distances) within populations are low accounting from 0.8 up to 1.7 % for the control region and 0.1 to 0.3 % in ND5/cyt b respectively (Table 16). Genetic divergence between the European and the Azorean populations ranges at approximately the same level at 1.75 % (cr) and 0.5 % (ND5/cyt b). TrN distances between the Canarian goldcrests and goldcrests from Europe and the Azores are much higher (3.3 %-4.2 % and 2.5 %-2.8 %, Table 16). Divergence of the two Canarian clades is also reflected by a high TrN distance of 3.4 % and 3.1 %. Highest distance values for the mitochondrial DNA were found between all western

palearctic goldcrest populations and the two Asian goldcrest subspecies, *R. r. tristis* and *R. r. japonensis* (for the control region and ND5/cyt b gene 3.9 %-5.2 % and 3.6 %-5.9 %, respectively, Table 16).

Table 16 TrN distances between goldcrest populations from w Europe, the Atlantic islands and Asia for the investigated fragment of the control region [1] (below diagonal) and partial ND5-cytochrome *b*-genes (above diagonal)
Grey shaded cells: distances within populations as inferred from control region (ND5/cyt *b*-) sequences. Canary Islands: T= Tenerife, LG = La Gomera, EH = El Hierro, LP = La Palma.

	Europe	Azores	T/LG	EH/LP	*tristis*	*japonensis*
Europe	**0.001** 0.009	**0.005**	**0.0279**	**0.025**	**0.036***	**0.048***
Azores	0.018	**0.001** 0.010	**0.0288**	**0.026**	**0.040***	**0.051***
Canary Isl. T/ LG	0.033	0.033	**0.0006** 0.0081	**0.031**	**0.036***	**0.054***
Canary Isl. EH/ LP	0.042	0.042	0.0347	**0.003** 0.006	**0.044***	**0.059***
R. r. tristis	0.050	0.052	0.0220	0.051	- 0.017	**0.036***
R. r. japonensis	0.043	0.050	0.0390	0.057	0.038	-

* based on 600 bp fragment of the mitochondrial cytochrome *b*-gene

[1] control region data contributed by M. Päckert and co-workers

Substitution rates and evolutionary age estimates

In the Likelihood Ratio Test the clocklike tree including all *Regulus* cr sequences was rejected on the $P < 0.05$ level (log L without clock = -1947.37; log L with clock = -1969.39), as was the clocklike tree including only the *R. regulus* sequences with *R. r. yunnanensis* as outgroup (log L without clock = -1400.98; log L with clock = -1419.83). Consequently, a constant substitution rate of the control region cannot be assumed within these two taxa groups. However, for a reduced data set including populations from Europe, the Azores and the Canarian El Hierro/La Palma group the clocklike tree is not rejected on the $P < 0.05$ level (log L without clock = -1375.44; log L with clock = -1386.07). For cyt b sequences the clocklike tree was also rejected for the complete data set (*R. regulus* ssp., *R. madeirensis*, outgroup *R. calendula*) on the $P < 0.05$ level (log L without clock = -3720.34; log L with clock = -3743.19). Only for a reduced data set including only the *R. regulus* ssp. haplotypes (*R. madeirensis* as an outgroup) the clocklike tree could not be rejected on the $P < 0.05$ level (log L without clock = -2810.44; log L with clock = -2820.98) confirming a constant substitution rate in the cytochrome b gene for Western Palearctic goldcrests.

As clocklike evolution is not corroborated for the entire taxonomic data set in both genes, input trees for r8s are not ultrametric. Therefore, nonparametric rate smoothing (NPRS) with POWELL was used, which relaxes the molecular clock assumption by using least square smoothing of local rates. Mean substitution rates estimated with r8s range at 0.004139-0.00443 substitutions per site and lineage per my for cytochrome b and 0.003059-0.00218 substitutions per site and lineage per my for the control region, respectively. These values roughly correspond to percentage substitution rates of 0.61-0.83 % between *Regulus* lineages per my. Rate variation among clades covers a broader range: 0-0.018 in cytochrome b (up to 3.6 % between lineages) vs. 0-0.0084 (up to 1.7 % between lineages) in the control region. Different clades of the two molecular trees show highest local rates: the Azorean clade in the control region data set (r_{loc}: 0.0064) and in the cyt b data set the Canarian clade and the E Asian *R. r. japonensis* clade (r_{loc}: 0.0081 and 0.0138, respectively; Table 15). Within the Canarian Archipelago, rate estimates for both genes are lower in the Tenerife/La Gomera clade (r_{loc}: 0.0017 and 0.0063, Table 15). Input files using simplified haplotype trees (one most common haplotype per taxon) yield considerably lower rate estimates for the cr data set and slightly higher estimates for cyt b data.

Evolutionary time estimates for the genus *Regulus* cover a broad range. The ancient split between the firecrest clade (*R. ignicapillus*/*R. madeirensis*) and the goldcrest clade (*R. regulus* /*R. satrapa*) is estimated about 48 my ago. Within the goldcrests, the Asian ssp. *yunnanensis* diverged from other *R. regulus* ssp. about 3.4 my ago. Colonization time estimates for the Atlantic islands range between 2.1-2.2 my ago for the first colonization of the Canary Islands (*R. r. teneriffae*), 1.8 my ago for the La Palma/El Hierro group and 0.7 my ago for the Azores (*R. r. azoricus, R. r. inermis*). The beginning of the inner-Azorean radiation approximately occurred 0.49-0.63 my ago.

Morphology

Canarian inter-insular morphological differences exist and are not even subtle in several respects. They are most obvious between males from the two genetically divergent island groups Tenerife/La Gomera and El Hierro/ La Palma, but homogeneous between the islands of the two groups. Females of all single islands or both island groups, respectively, do not show significant morphometric differences.

Tail length does not differ between islands and the two island groups, but differs between sexes independent of island origin. All males pooled: 37-40.5 mm (n = 21, mean = 39.1 ± 0.91 mm), all females pooled: 35.5-39.5 mm (n = 15, mean = 36.9 ± 1.0 mm). Wing length also does not differ between islands and the two island groups: all males pooled: 48-53.5 mm (n = 21, mean = 51.0 ± 1.1 mm); all females pooled: 48-52 mm (n = 15, mean = 49.1 ± 1.0 mm).

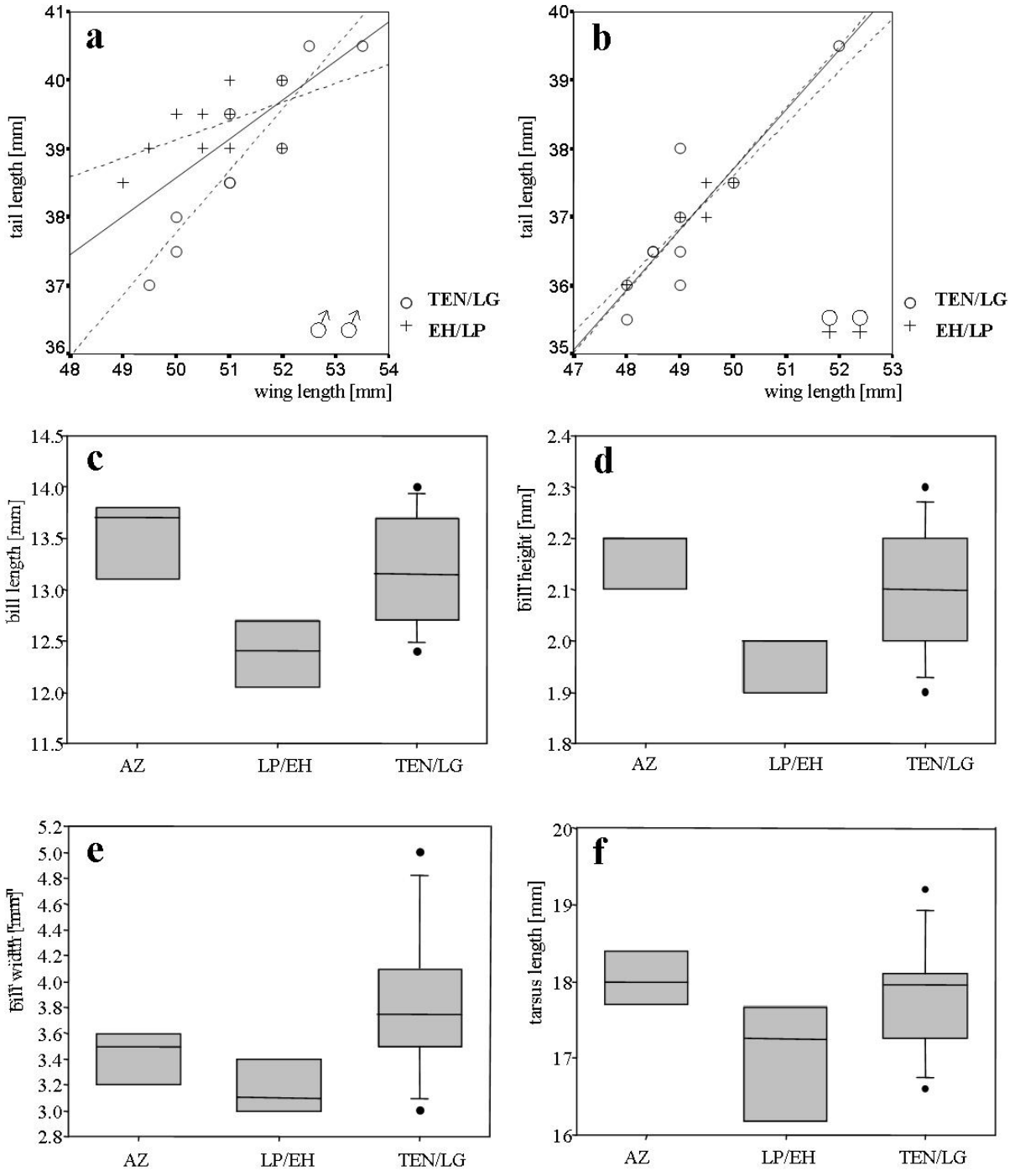

Figure 27 Morphological differentiation between Canarian populations of *R. r. teneriffae* (EH = El Hierro, LP = La Palma, TEN = Tenerife, LG = La Gomera)
Bars indicate mean values and standard error; a, b) wing-tail index; c-d) bill length, height and width; f) tarsus length.

Tail-wing index (Figure 27a, b): in males (not females) significantly differ between the two island groups (El Hierro/La Palma: n = 11, mean = 0.776 ± 0.012; Tenerife/La Gomera: n = 10, mean = 0.759 ± 0.010; Mann Whitney-U test, $P < 0.05$).

Bill length: significantly different in males (not in females) in the two island groups (El Hierro/La Palma: n = 11, mean = 11.3 ± 0.39 mm; Tenerife/La Gomera: n = 10, mean = 11.7 ± 0.52 mm; Mann Whitney-U test, $P < 0.05$).

Tarsus length does neither differ between the two island groups, but differs significantly between sexes on Tenerife/ La Gomera (Mann Whitney-U test, $P < 0.05$). All males pooled: 16.4-18.6 mm (n = 21, mean = 17.7 ± 0.66 mm), all females pooled: 16.4 - 18.1 mm (n = 15, mean = 17.2 ± 0.57 mm).

Further significant differences between sexes concern the length of wings, tails on both island groups (Mann Whitney-U test, $P < 0.05$) and in addition on El Hierro/ La Palma the parameters tail-wing index and bill length (Mann Whitney-U test, $P < 0.05$). In live birds morphological differences between sexes were less developed but might be underestimated since sample sizes were low. Significant differences (Mann Whitney-U test) between island groups (all birds of both sexes pooled) were found in bill height (Figure 27c; El Hierro/La Palma: n = 7, mean = 2.0 ± 0.1 mm; Tenerife/LaGomera: n = 12, mean = 3.9 ± 0.5 mm; $P < 0.01$) and tarsus length (Figure 27f; El Hierro/La Palma: n = 8, mean = 17.0 ± 0.9 mm; Tenerife/La Gomera: n = 12, mean = 17.8 ± 0.7 mm; $P < 0.05$; Table 17). Crown stripes are slightly divergent in males of the two island groups. The stripes in the Tenerife/ La Gomera group are markedly more intensely orange than in males from El Hierro and La Palma. Crown stripes of males in the latter group are paler and show a more yellowish tinge though orange prevails.

Table 17 Morphometric measurements of *Regulus ssp.* from live birds captured on the Canary Islands (n = 30) , Madeira (n = 16) and the Azores (n = 7)
Abbrevations: P = primary, S = secondary, NaLoSpi = distance from distal edge of nostril to bill tip.

	Azores			La Palma/El Hierro			La Gomera/Tenerife			Madeira		
	mean	s.d.	range	mean	s.d.	range	mean	s.d.	range	mean	s.d.	Range
Body Mass [g]	6.0	0.4	5.3-6.4	5.2	0.7	4.4-6.8	5.4	0.4	4.5-6.2	6.4	0.5	5.3-6.8
Wing [mm]	53.0	1.2	52.0-55.0	50.6	1.2	49.0-52.0	51.4	1.3	48.0-53.0	54.9	1.7	52.0-57.0
P9 [mm]	35.9	1.8	33.5-39.0	32.9	2.0	29.5-35.0	34.7	1.8	32.0-39.0	35.0	1.4	32.0-37.0
P8 [mm]	41.6	1.3	39.5-44.0	39.0	1.7	36.0-41.0	40.2	1.1	37.5-42.0	42.1	1.4	39.5-43.5
P7 [mm]	43.8	1.3	42.0-46.0	41.3	1.3	39.5-43.0	41.6	1.3	38.5-43.5	44.5	1.6	41.5-47.0
P6 [mm]	44.6	1.3	43.0-47.0	42.2	1.4	40.5-44.5	42.7	1.1	41.5-45.0	46.0	1.6	43.0-48.5
P5 [mm]	44.9	1.1	43.5-46.5	42.8	1.3	41.0-44.5	42.9	1.2	40.5-44.5	46.5	1.5	43.5-49.0
P4 [mm]	42.9	0.9	42.0-44.5	41.0	1.1	39.5-42.5	41.3	1.1	39.0-43.0	44.8	1.4	42.0-47.0
P3 [mm]	41.6	0.9	40.5-43.0	39.7	1.3	38.0-41.0	40.1	0.9	38.0-41.5	43.3	1.3	40.5-45.0
P2 [mm]	40.8	1.0	39.5-42.5	39.0	1.1	37.5-40.5	39.5	0.9	37.5-40.5	42.3	1.2	39.5-44.0
P1 [mm]	39.9	1.0	38.5-41.5	37.9	1.6	34.5-39.0	38.5	0.8	37.0-39.5	41.5	1.1	39.0-43.0
S1 [mm]	39.4	1.4	38.0-41.5	38.0	1.0	36.5-39.0	38.6	0.9	37.5-40.0	41.5	1.3	39.0-43.0
Tarsus [mm]	18.0	0.3	17.6-18.5	17.0	0.9	15.6-18.2	17.8	0.7	16.6-19.2	19.6	0.7	18.4-21.2
NaLoSpi [mm]	6.4	0.3	6.2-6.9	6.3	0.3	5.8-6.7	6.5	0.3	6.1-6.9	6.2	0.7	5.0-7.1
Bill width [mm]	3.5	0.3	3.2-4.0	3.2	0.2	3.0-3.5	3.9	0.5	3.0-5.0	3.7	0.3	3.2-4.3
Bill length [mm]	13.5	0.4	13.0-14.0	12.4	0.3	12.0-12.9	13.2	0.5	12.0-13.8	13.3	0.8	12.0-14.6
Bill height [mm]	2.2	0.1	2.0-2.4	2.0	0.1	1.8-2.1	2.1	0.1	1.9-2.3	2.3	0.1	2.2-2.5

Vocalizations[iii]

Territorial song of Canarian goldcrests shows the typical bipartite structure of *R. regulus* with a stereotype main part and a variable terminal flourish (Figure 28a). Males of the entire Canarian population share one common song type A, and this song type is homogeneous throughout the four islands inhabited by the species. It begins with a short trill, a repetition of a single short element, which is followed by a few call-like notes (Figure 28c-g, l). Slight inter-individual variation can be detected in frequency modulation of trill elements. In the sonagram some of them are hooks opened upwards (v-shaped, Figure 28c-d) or downwards (Figure 28e-f) or are even barely modulated (Figure 28g). Every recorded male from the Canary Islands displayed this song type. Songs of individual males may also include double trills (Figure 28c). On the Azores, this song type is common on the Eastern islands São Miguel and Santa Maria (Figure 28b). Apart from that widespread song type, most males from La Palma and El Hierro possess at least one other song type, which differs from type A in element composition. In these songs the trill part is shortened, of a low amplitude or even lacking. Males from La Palma display a song type B, which includes a long series of variable strongly modulated elements in a fixed order (Figure 28h, i). There is slight inter-individual variation in order and shape of elements. Males from El Hierro display a third song type C, which includes fixed groups of elements (motifs) which are repeated once (motifs I and II; Figure 28k, m). Due to the alteration of different elements this song type is more rhythmic than types A and B. Song type B is restricted to La Palma, while song type C was exclusively found on El Hierro. On La Gomera only song type A was recorded from nine males. On Tenerife individual song types with rhythmic groups were recorded from several males of the Anaga mountains population (not shown). However, these song types are of a different syntax and element structure than those from El Hierro and La Palma.

[iii] Contributed by M. Päckert and Co-workers

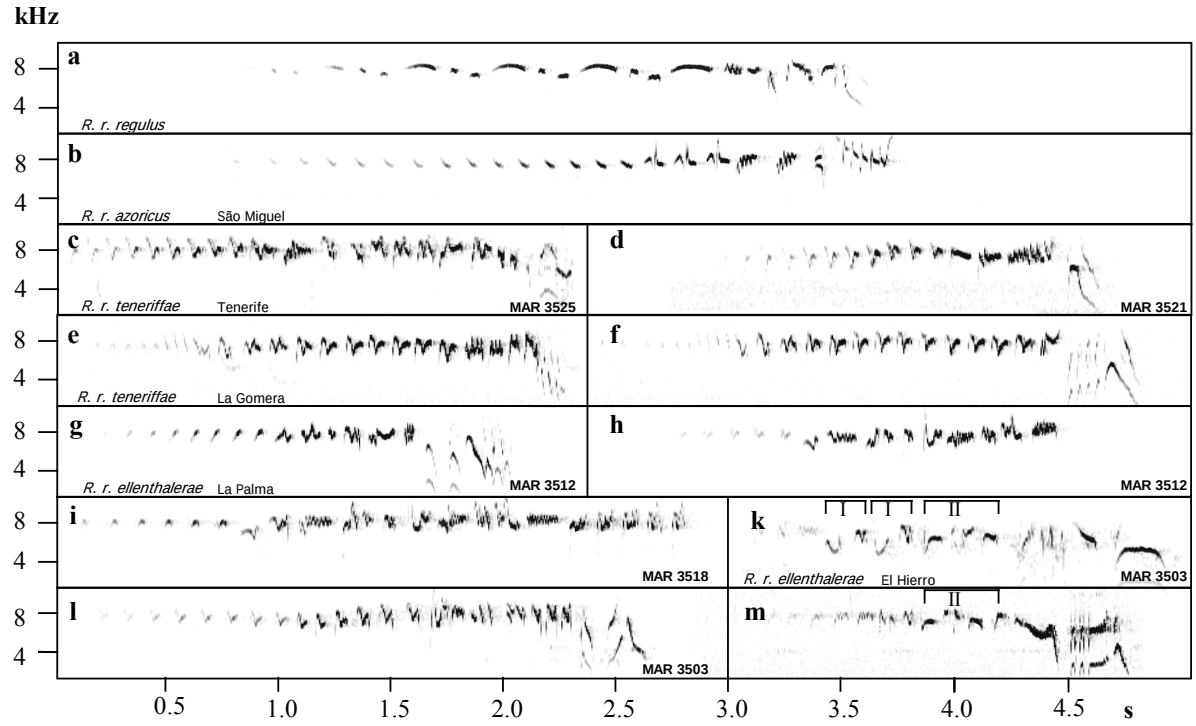

Figure 28 Differentiation of territorial song between western palearctic goldcrest subspecies and among Canarian goldcrest populations
All recordings by M. Päckert 2000-2003: a) *R. r. regulus*: Germany, Taunus; b) *R. r. azoricus*: Azores, São Miguel (type A); *R. r. teneriffae*: c), d) two males from Tenerife (type A), e), f) two strophes of one male from La Gomera (type A); *R. r. ellenthalerae* n. ssp., La Palma: g), h) male MAR3512 (type A and type B), i) male MAR3518 (type B); *R. r. ellenthalerae* n. ssp., El Hierro: k), l) male MAR3503 (type C and type A), m) another male, type C; fixed groups of elements (motifs) indicated by Roman numbers I and II.

3.3.4 Discussion

Goldcrest radiation on the Atlantic islands

According to the molecular data the Atlantic islands were settled by goldcrests within two major events: an early invasion to the Canary Islands 2.1-2.2 my ago and a more recent one to the Azores 0.7 my ago. A single Atlantic colonization event followed by a stepwise radiation from the Canaries to the Azores is not supported by the data presented here - the topology suggesting monophyly of the entire Atlantic goldcrest populations is the only scenario which is significantly rejected by SH and KH tests. Two most common haplotypes represent two major founder events within the latter archipelago: colonization of the eastern part of São Miguel and Santa Maria (haplotype G) and a second colonization of all remaining western islands (haplotype F, including the western crater of São Miguel). Haplotypes J, K and L represent two minor lineages of radiation. Distribution of goldcrest haplotypes supports a division of the Azorean metapopulation into an eastern and a western group as already inferred from song dialects (Päckert & Martens 2004). Acoustic and molecular findings also point to a substructuring of *R. r. azoricus* on São Miguel into

an eastern population (Furnas) with close affinities to *R. r. sanctaemariae* and a western population (Sete Cidades) as the initial point of the radiation to the central and western group (common haplotypes with *R. r. inermis* ; see also Päckert et al. 2003). The low pairwise ΦST-value between the eastern *R. r. azoricus* and the western *R. r. inermis* is certainly due to the shared haplotype F and might reflect past gene flow between these populations due to past population expansion. The latter is also indicated by significant raggedness index for *R. r. azoricus*. The Azorean populations have always been considered recent descendants from continental European goldcrests (Volsoe 1951, Päckert et al. 2003) while the latter are supposed to have undergone a recent post-pleistocene expansion from southeastern or Asian refuge areas (Salomonsen 1930, Becker 1978). Common ancestry of European and Azorean goldcrests is evident from molecular data, however, the interpretation of migration rates is crucial for these two populations.

According to the considerably higher migration rate from the Azores to Europe compared to the opposite direction, one might assume that the Azorean archipelago could have served as a further pleistocene refuge area - along with southeastern continental areas - from which goldcrests re-colonized the European continent after the pleistocene. However, the asymmetry of the values between Europe and the Azores might also be due to high frequency of haplotype F in a relatively low sample size, giving more weight to that population as a source. Furthermore, migration rates between any other pair of goldcrest populations are considerably low - in most cases below one individual per generation and rather point to complete isolation than to recent gene flow. Especially for the major clades of the western Palearctic goldcrests time estimates confirm a long time span of isolate evolution during the last 2 million years. Colonization events on the southern Atlantic archipelagoes occurred during the late Pliocene period. The ND5/cyt b data confirm that the population on Madeira, *R. madeirensis*, forms a distantly related sister group to European firecrests as already suggested earlier (Päckert et al. 2003). For the Canary Islands, population substructuring indicated by bimodal mismatch distributions and molecular phylogenetic reconstructions suggest a two-step colonization: A first invasion to the northeastern group (Tenerife and La Gomera; 1.9-2.3 my ago) and a second one to the southwestern islands, La Palma and El Hierro, at a later period (1.3-1.8 my ago). However, monophyly of the Canarian populations is not significantly rejected by SH and KH tests, so a single invasion of goldcrests to Canarian archipelago cannot be excluded on ground of molecular data. In several passerine species the Canarian metapopulation shows a similar genetic structure to that found in the goldcrests. Chaffinches from El Hierro (*Fringilla coelebs ombriosa*) and La Palma (*F. c. palmensis*) form a separate monophyletic sister group to a complex of all remaining Canarian populations (Marshall & Baker 1999). Likewise in Canarian blue tits, the population from La Palma (*Parus teneriffae palmensis*) was shown to be a separate clade deeply divergent clade from other Canarian subspecies

(Kvist et al. 2005). Finally, the Canarian robins (*Erithacus rubecula* ssp.) are genetically split into three separate populations, one each endemic to Tenerife and to Gran Canaria and a third one coinciding with mainland Europe (Dietzen et al. 2003).

Rates of molecular evolution

To date, the number of reliable calibrations of molecular clocks in avian taxa is extremely small and meanwhile the results became a standard for ornithological molecular systematics. Recently, these calibrations have been critically revised (Garcia-Moreno 2004, Lovette 2004) with particular respect to the frequently adopted ‘‘2 % rule’’. This commonly accepted approximation to the substitution rate per my of the cytochrome b gene resulted from a study in geese (Shields & Wilson 1987). A calibration for Hawaiian Honeycreepers (Fleischer et al. 1998) yielded a slightly lower value of 1.6 % per my and offers a most adequate comparison to the rate estimates for the Atlantic goldcrests, because both studies refer to passerine taxa and both basically refer to paleogeographic data as calibration points instead of fossil records. Though the estimate by Fleischer et al. 1998) notably exceeds mean rate estimate for cytochrome b in *Regulus* (0.83 % per my), it falls into the large range of local rate variation within the cyt b data set from 0.012 % up to 2.4 % between *Regulus* clades. Estimates based on the reduced "most common haplotype" trees (which like in the study by Fleischer et al. 1998) include only a single tip clade for each taxon), yielded lower values, and consequently result in an underestimation of mean substitution rates of both cytochrome b and the control region. Different evolutionary rates among and within avian lineages were recorded in various taxa for different mitochondrial genes and are a most convincing argument against a universal substitution rate of 2 % per my in birds (Ruokonen & Kvist 2002). Conclusively, rate estimates for both mitochondrial data sets of *Regulus* (0.61-0.83% per my) hold against a generally faster rate of the control region vs. cytochrome b as confirmed in former studies: *Phylloscopus* (Irwin et al. 2001), *Fringilla coelebs* (Baker & Marshall 1997), and *Parus major* (Päckert et al. 2005). A recent review of avian sequence data confirms a wide range of substitution rates between 0.13 and 21.7 % for the control region (Ruokonen & Kvist 2002). Compared to the continental population of nominate *R. r. regulus*, fastest local rates were observed in island clades (Azorean, partly the Canarian and the *japonensis* subspecies group). On the other hand, local rates in the Tenerife/La Gomera clade range at approximately the same level (cyt b) or even at a much lower level (cr) than in the continental clade, thus there is only mixed support for generally faster rates in island goldcrest lineages from data presented here. Increased rates of cytochrome b and ND2 due to an accumulation and fixation of non-synonymous substitutions were also confirmed for island populations of dabbling ducks (genus *Anas*) and doves (genus *Zaidura*) and might have resulted from repeated bottleneck effects in small populations (Johnson 2001). However, among 19 vertebrate and

invertebrate taxa Bromham & Woolfit (2004) did not find evidence for an acceleration of a molecular clock due to explosive adaptive radiations on islands. In Atlantic goldcrests, the relatively low N_{fe} estimates for the Azorean (and partly the Canarian) population indicate that fluctuations of population size could have had an accelerating impact on substitution rates in island *Regulus* lineages. The high rate estimate for the *R. r. japonensis* clade matches that interpretation because this subspecies encompasses many island populations of Japan and Sakhalin, and presumably was restricted only to relict coniferous habitats on these islands during cold periods of the late pleistocene (Nazarenko 1990). On the other hand, enhanced drift in small populations might decrease genetic variation and thus lead to an underestimation of substitution rates, i.e. in the Tenerife/ La Gomera group (low rate of the control region).

Taxonomic implications

Most passerine species on the Canary Islands differ considerably from their mainland European relatives and, in addition, have often undergone marked interinsular differentiation. The main emphasise of this study lies not on the taxonomic status of the entire Canarian goldcrest population and to the controversy whether the ‘‘Tenerife goldcrest’’ merits species status or not. For reasons of morphological, ethological and genetic diagnosibility it was considered a separate species (Seebohm 1883, Löhrl & Thaler 1980, Sturmbauer et al. 1997), but was also treated as a subspecies of *R. regulus* on the basis of bioacoustic analyses (Becker 1978, Martens et al. 1998), and of cross-breeding experiments in captivity (Löhrl et al. 1996). For the high intraspecific differentiation of *R. regulus* on a genetic and bioacoustic level, a taxonomic discussion is only recommended with respect to the even more divergent Asian goldcrest subspecies *R. r. tristis*, *R. r. japonensis* and *R. r. himalayensis/ yunnanensis* as already done earlier (Martens & Päckert 2003).

Morphological goldcrest variation is well documented for the Azores expressed by three nominal subspecies which were described for the archipelago (measurements are given in Cramp & Perrins 1993). But intra-Canarian goldcrest variation escaped notice ever since and no data for individual islands exist. Vaurie (1954) and Cramp & Perrins (1993) present data combined from material of all islands, and most of the skins used had been taken also for this study. Marked variation occurs between males of the two island groups, Tenerife/La Gomera and El Hierro/La Palma, which are characterized by the deep split of the genetic markers. Koenig (1890) already noticed the deep-orange coloration of the male’s crown stripe ("hochorangerothe Scheitelfärbung") of his *Regulus satelles* from Tenerife (figured by him in 1890 on colour plate V, Fig. 1), but interinsular variation escaped his notice. There is no general inter-insular variation covering all islands separately. Coloration of crown stripe, male wing-tail-index and bill measurements vary

always according to the two island groups. Long separate history of the two island groups is corroborated also by these morphological characters. Traditionally, these differences are strong enough to warrant subspecific separation.

However, the molecular analysis provides evidence for a new goldcrest taxon from the Canarian archipelago on the islands of El Hierro and La Palma. These populations are genetically divergent at the same distance level from goldcrests of Tenerife and of La Gomera on the one hand and from Central European and Azorean populations on the other hand. The type locality of "*R. teneriffae*" (Seebohm 1883) was never restricted to any specific island, only "Canary Islands" was indicated in the original description. On grounds of the molecular and morphological data presented, a split of the Canarian goldcrests into two subspecies seems justified: *Regulus regulus teneriffae* Seebohm, 1883 on Tenerife and La Gomera and a second one from La Palma and El Hierro. For the taxon from the latter two islands the name *Regulus regulus ellenthalerae* nov. ssp. is suggested.

Description of a new subspecies

Regulus regulus ellenthalerae nov. ssp. Diagnosis: A subspecies of the goldcrest (*Regulus regulus*) from the islands of La Palma and El Hierro, Canary Islands, Spain, characterized by light yellow-orange crown stripe in males (deep reddish-orange in *R. r. teneriffae*, the latter similar to mainland *R. ignicapillus*), finer bill in males, higher tail/wing index in males (as opposed to *R. r. teneriffae*), and a significant cluster of mitochondrial control region and ND5/cytochrome b haplotypes, sequences AY664566-AY664579 and AY894837-AY894840, GenBank (as opposed to *R. r. teneriffae*, *R. r. regulus*, *R. r. tristis*, *R. r. yunnanensis* and *R. r. japonensis*).

Material: Holotype: GX6a3sigma, La Palma, Hoya Grande; Jan. 1905, R. v. Thanner: wing 50.5 mm, tail 39 mm, tarsus 16.6 mm, bill 10.9 mm, wing/tail index 0.772. Paratypes: GX6a3.2xgamma, El Hierro, Pinar; Feb. 1905, R. v. Thanner leg.: wing 51 mm, tail 39.5 mm, tarsus 17 mm, bill 10.9 mm, wing/tail index 0.775. GX6a3.2xkappa, El Hierro, Pinar; Feb. 1905, R. v. Thanner leg.: wing 52 mm, tail 39 mm, tarsus 17 mm, bill 12.2 mm, wing/tail index 0.75. Additional material: La Palma, six males, three females; El Hierro: two males, two females of the collection of the Museum A.Koenig, Bonn, Germany. Comparison material of *R. r. teneriffae* (now restricted to the islands of Tenerife and La Gomera): Tenerife: four males, four females; La Gomera: six males, six females.

General description: outer morphology of *R. r. ellenthalerae* nov. ssp., especially allover appearance including coloration is very similar to *R. r. teneriffae*, but differences in male crown stripe coloration is striking, even in individual specimens, but direct comparison is recommended. Underparts of *R. r. ellenthalerae* are less brownish than in *R. r. teneriffae* in both sexes.

Morphology: wing: 49-52 mm in males (mean = 50.7 ± 0.9 mm, n = 11), 48-50 mm in females (mean = 49.2 ± 0.8 mm, n = 5); tail: 38.5-40 mm in males (mean = 39.3 ± 0.5 mm), 36-37.5 mm (mean = 37 ± 0.6 mm) in females; tail/wing index of males: mean = 0.776 (0.759 in males [n = 10] of *R. r. teneriffae*, $p < 0.05$); bill: 10.9-11.7 mm (mean = 11.3 ± 0.4 mm, 11.7 ± 0.5 in *R. r. teneriffae*, $P < 0.05$); tarsus: 16.4-18.5 mm in males (mean = 17.6 ± 0.7 mm), 16.4-18.1 mm in females (mean = 17.2 ± 0.7 mm). Live birds of *R. r. ellenthalerae* (both sexes) showed the following measurements: wing: 49-52 mm (mean = 50.6 ± 1.2 mm, n = 8), P9: 29.5-35.0 mm (mean = 32.9 ± 2.0 mm, n = 8), P8: 36.0-41.0 mm (mean = 39.0 ± 1.7 mm, n = 8), P7: 39.5-43.0 mm (mean = 41.3 ± 1.3mm, n = 8), P6: 40.5-44.5 mm (mean = 42.2 ± 1.4 mm, n = 8), P5: 41.0-44.5 mm (mean = 42.8 ± 1.3 mm, n = 8), P4: 39.5-42.5 mm (mean = 41.0 ± 1.1 mm, n = 8), P3: 38.0-41.0 mm (mean = 39.7 ± 1.3 mm, n = 8), P2: 37.5-40.5 mm (mean = 39.0 ± 1.1 mm, n = 8), P1: 34.5-39.0 mm (mean = 37.9 ± 1.6 mm, n = 8), S1: 36.5-39.0 mm (mean = 38.0 ± 1.0 mm, n = 8), tarsus: 15.6-18.2 mm (mean = 17.0 ± 0.9 mm, n = 8), NaLoSpi: 5.8-6.7 mm (mean = 6.3 ± 0.3 mm, n = 7), bill width: 3.0-3.5 mm (mean = 3.2 ± 0.2 mm, n = 7), bill length: 12.0-12.9 mm (mean = 12.4 ± 0.3 mm, n = 8), bill height: 1.8-2.1 mm (mean = 2.0 ± 0.1 mm, n = 7). For comparative values of *R. r. teneriffae* see Table 17. For further measurements of Canarian goldcrests without insular affiliation see Cramp & Perrins (1993).

Vocalisations: Territorial song in both subspecies is similar on all islands, but differences exist: As in all *R. regulus* subspecies it is partitioned into a stereotype main part and a variable terminal flourish; one song type which occurs in common with *R. r. teneriffae* consists of a rapid trill of short notes ending in a few call like notes plus the terminal flourish (Figure 28c-f, g, l). In addition to this wide-spread song type, *R. r. ellenthalerae* nov. ssp. disposes of additional song types (Figure 28h-k, m): The main part consists of a series of differently modulated and variable elements of a fixed and often irregular order. Elements may be combined to fixed groups, which are repeated once; structure is more rhythmic than in songs of *R. r. teneriffae*.

Etymology: This clearly characterized and distinct insular subspecies is named in honour of Dr. Ellen Thaler, Innsbruck, who contributed so much to our knowledge on biology, behaviour, ecology and systematics on *Regulus* species. Her longlasting work on captive birds is unique and may hardly be paralleled.

Nomenclature: Seebohm (1883) in his description of "*Regulus teneriffae*" did not restrict the type locality to any of the Canary Islands. Alluding to the specific epithet, the type locality of the taxon *teneriffae* is herewith restricted to the island of Tenerife, Canary Islands. In addition, *teneriffae* occurs on La Gomera. Koenig (1889) produced another name for Canarian goldcrests, *Regulus satelles*. As the type locality he gives Tenerife and,

consequently, the name is not available for the differing populations of La Palma and El Hierro. Already Hartert (1907) put *satelles* Koenig in the synonymy of *teneriffae* Seebohm.

Distribution: On the Canarian archipelago *Regulus regulus ellenthalerae* is restricted to the southwestern islands La Palma and El Hierro. There, goldcrests occupy the same ecological niche as does *R. r. teneriffae* on Tenerife and La Gomera (Martin & Lorenzo 2001). On El Hierro *R. r. ellenthalerae* is common in the laurel forests, El Brezal and El Golfo and in small forest areas near Valverde up to 700 m, while none were observed in the pine forests of El Pinar. In contrast, on La Palma *ellenthalerae* goldcrests were found also in pine forests of the central mountain massif La Cumbre up to 2000 m. Their occurrence is certainly due to the presence of *Erica arborea* bushes in this area, which are lacking in El Pinar (El Hierro).

3.4 Phylogeography of the blue tit (*Parus teneriffae* – group) on the Canary Islands

3.4.1 Introduction

Within the Paridae, the blue tit complex and two closely related species, the Azure tit (*P. cyanus*) and the yellow-breasted Tit (*P. flavipectus*), have been subsumed under the subgenus *Cyanistes* (Harrap & Quinn 1996). Some authors conclusively recommended the elevation of six parid genera from the respective subgenera including *Cyanistes* (Gill et al. 2005). In this study the traditional taxonomy (Harrap & Quinn 1996) is followed until a broad and thorough review of the Paridae including morphological and bioacoustic markers is available as well as a more exhaustive taxon sampling including subspecies.

The polytypic *P. caeruleus* is distributed over large parts of Europe from Macaronesia to the Ural Mountains including also North Africa and Asia Minor (Cramp & Perrins 1993, Glutz von Blotzheim & Bauer 1993). Traditionally at least 15 subspecies are recognised with nominate *caeruleus* in northern, central and eastern Europe south to northern Spain, Italy, Greece and Asia Minor, *P. c. ogliastrae* in southern Iberia, Corsica and Sardinia, *P. c. ultramarinus* in northwestern Africa and the four Canary Island taxa *ombriosus* (El Hierro), *palmensis* (La Palma), *teneriffae* (La Gomera, Tenerife, Gran Canaria) and *degener* (Fuerteventura, Lanzarote) to name just a few (Dickinson 2003). Recent molecular studies gave evidence for splitting the northern *caeruleus*–group (Europe) from the southern *teneriffae*–group, including *ultramarinus* and the Canary Island taxa (Salzburger et al. 2002a, Kvist et al. 2004), which is also applied in this study. The same authors have furthermore suggested conspecificity of *P. cyanus* and *P. flavipectus*. The validity of most Canary Island taxa (*palmensis, ombriosus, degener*) was confirmed by sequence data of the mitochondrial control region, while *teneriffae* includes two distinct genetic lineages (Kvist et al. 2005).

Sequences of the mitochondrial cytochrome b–gene were used to study the phylogeographic differentiation of the blue tits on the Canary Islands and to test the phylogenetic relationships of the taxa involved, in particular the validity of the (sub-) specific differentiation of taxa as proposed by different authors (e.g. Kvist et al. 2005). A further objective concerns the colonization history of the blue tit in the Canarian Archipelago.

3.4.2 Material and Methods

Samples

The samples for this study (n = 63) were obtained from live birds on the Canary Islands in 2002-2005 (Appendix A). The birds were captured with mist-nets, measured, weighed and blood samples were obtained by puncturing the brachial vein. Afterwards the birds were released and the blood samples were preserved in storage buffer containing 0.1 M Tris, pH 7.4, 10 % EDTA, 1 % NaF, 0.1 % thymol and frozen at –20 °C as soon as possible until further processing.

Sequencing

Details of DNA extraction, gene amplification and sequencing reactions are described in chapters 3.1.2 and 3.2.2. The mitochondrial cytochrome b-gene was amplified by PCR from the total genomic DNA using the primers 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').

The 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 mt-A1, mt-E (H15700; 5'-GAT GGC GTA GGC AAA TAG GAA GTA TCA TTC TGG TTT-3') 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.

Phylogenetic Analysis

By using different primer combinations, overlapping sequences with a combined length of 1 005 nucleotides were obtained from 63 blue tits and one great tit (outgroup). Sequences were aligned and net pairwise genetic p-distances calculated with MEGA version 2.1 Kumar et al. 2001). Phylogenetic trees were constructed employing PAUP*4b10 - Neighbour-Joining, Maximum Parsimony and Maximum Likelihood – (Swofford 2001) and MrBayes 2.01 (Huelsenbeck & Ronquist 2001).

An appropriate substitution model for the maximum likelihood calculations was estimated via likelihood ratio test with Modeltest 3.04 (Posada & Crandall 1998). The selected model was a modification of the Hasegawa-Kishino-Yano (HKY85) model TVM+I+G (Posada & Crandall 1998). Likelihood settings were as follows: empirical base frequencies pA = 0.2799, pC = 0.3711, pG = 0.1259, pT = 0.2231; substitution rates $R_{[A–C]}$ = 1.9577, $R_{[A–G]}$ = 17.1739, $R_{[A–T]}$ = 0.4010, $R_{[C–G]}$ = 0.0000, $R_{[C–T]}$ = 17.1739, $R_{[G–T]}$ = 1.0000 and gamma distribution shape parameter α = 0.3862. A minimum spanning network was constructed employing TCS 1.13 (Clement et al. 2000). Nucleotide diversity, π (Nei

1987), haplotype diversity ĥ (Nei 1987), θ (Tajima 1996) and mismatch distributions including raggedness stastic (Harpending 1994) were calculated with DnaSP v. 3.51 (Rozas & Rozas 1999). Genetic structure was evaluated using analysis of molecular variance (AMOVA) employing Arlequin v. 2.0 (Excoffier et al. 1992). Several assumed genetic structures were tested (Table 20).

Morphometrics

All birds captured for sampling (n = 76) were measured and weighed. The following measurements were taken as described (Svensson 1992): maximum wing length, length of primaries (P) 1-9 and secondary (S) 1, length of tarsus (bent), length of bill tip to distal end of nostril (NaLoSpi), bill width, bill depth and bill length from tip to skull. 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 $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***). To investigate possible morphological differentiation between populations the data were entered into a discriminant function analysis (Wilk's Lambda). Only adult birds not in moult were included.

3.4.3 Results

Molecular phylogeography

The obtained sequences (1 005 base pairs) could be aligned without difficulties and no stop codons were encountered. The employment of different primers produced overlapping sequences, which gave some additional proof that sequences were correct and of mitochondrial origin.

1 005 nucleotides in the *Parus* dataset showed 110 (10.9 %) variable sites of which 108 (10.7 %) were parsimony informative (Appendix D). The sequences could be assigned to 29 different haplotypes and the haplotype diversity was 0.963 for the complete data set. Nucleotide diversity for all *Parus* sequences was 0.02746. Considering only samples from the Canary Islands haplotype diversity and nucleotide diversity were lower, h' = 0.954 and $\pi = 0.02161$, respectively. Sequences from Europe showed a much lower nucleotide diversity ($\pi = 0.00518$) but sample size was low for the latter. The same applies for θ values with $\theta = 0.01619$ in the Canary Islands and $\theta = 0.00479$ in Europe. Further diversity indices are listed in Table 18. Each of the Canary Island haplotypes was confined to just one single island although the number of haplotypes varied between islands, in part

influenced by sample sizes. The largest haplotype diversity was found on Gran Canaria followed by Tenerife (Table 18).

Table 18 DNA polymorphismn in the mitochondrial cytochrome b gene of different populations of *Parus teneriffae/caeruleus* on the Canary Islands

Population	N	Number of haplotypes	Haplotype diversity	Nucleotide diversity	θ	Tajima's D	
Tenerife	8	5	0.85700	0.00150	0.00115	1.21973	n.s.
La Gomera	10	4	0.73333	0.00151	0.00211	-1.18950	n.s.
Gran Canaria	9	6	0.91667	0.00233	0.00183	1.13663	n.s.
El Hierro	6	2	0.33333	0.00033	0.00044	-0.93300	n.s.
La Palma	5	2	0.40000	0.00239	0.00287	-1.14550	n.s.
Fuerteventura	9	3	0.72200	0.00094	0.00073	0.97505	n.s.
Lanzarote	7	1	0.00000	0.00000	0.00000	-	
Morocco	4	1	0.00000	0.00000	0.00000	-	
Europe	5	5	1.00000	0.00518	0.00479	0.59633	n.s.

The net pairwise genetic p-distances between Canary island populations and European samples ranged from 5.9 to 6.8 %. The variation within (island) populations was low (0.0 to 0.5 %). Genetic distances between island and neighbouring mainland populations suggest some unexpected groupings (Table 19), which are also evident from the tree topologies obtained through phylogenetic sequence analyses (Figure 29). Tree topologies were identical for all tree-building methods applied. Opposed to the samples from Europe the samples from the Canary Islands formed a monophyletic group, which also includes birds from North Africa (Morocco) displaying much smaller genetic distances to Canary island populations, particularly to Fuerteventura and Lanzarote (0.2-0.3 %). At the base of the Canarian clade samples from La Palma (*P. c. palmensis*) clustered as a sister taxon to the remaining island populations. In the latter group several clusters can be distinguished from base to top with 1) samples from the eastern Canary Islands (Fuerteventura, Lanzarote) together with those from Morocco (*P. c. degener, P. c. ultramarinus*), 2) birds from El Hierro (*P. c. ombriosus*), and 3) two sister groupings with birds from Tenerife and La Gomera on the one hand and birds from Gran Canaria on the other hand (*P. c. teneriffae*). Genetic distances between the latter were 1.1 % while other island taxa displayed sequence divergences from 2.3 to 3.4 %. All Canary island taxa are monophyletic.

Table 19 Genetic net pairwise p-distances and pairwise Φ_{ST} values in the mitochondrial cytochrome b gene between *Parus teneriffae/caeruleus* populations of the Canary Islands
Net p-distances below diagonal [%], within group distances in the diagonal (bold) and pairwise Φ_{ST} values above diagonal. n.d. = not determined.

	[1] La Palma	[2] El Hierro	[3] La Gomera	[4] Tenerife	[5] Gran Canaria	[6] Fuerte-ventura	[7] Lanza-rote	[8] Morocco	[9] Europe
[1]	**0.2**	0.971	0.952	0.952	0.942	0.961	0.972	0.971	n.d.
[2]	4.8	**0.0**	0.959	0.960	0.945	0.981	0.995	0.995	n.d.
[3]	4.0	2.3	**0.2**	0.637	0.831	0.956	0.972	0.970	n.d.
[4]	4.1	2.3	0.4	**0.1**	0.830	0.954	0.971	0.969	n.d.
[5]	4.1	2.4	1.1	1.1	**0.2**	0.931	0.950	0.945	n.d.
[6]	4.3	3.4	2.8	2.7	2.4	**0.1**	0.787	0.823	n.d.
[7]	4.3	3.3	2.7	2.6	2.3	0.2	**0.0**	1.000	n.d.
[8]	4.1	3.1	2.5	2.4	2.1	0.3	0.2	**0.0**	n.d.
[9]	5.9	6.2	6.1	6.1	6.4	6.8	6.7	6.5	**0.5**

Table 20 Analysis of Molecular Variance (AMOVA) for different definitions of Canary Island *Parus* – taxa.

	Variation among defined taxa	Variation among islands	Variation within islands	Φ_{SC}	Φ_{ST}	Φ_{CT}
5 Taxa[1]	76.4%	19.3%	4.3%	0.81785	0.95700	0.76396
5 Taxa[2]	87.7%	7.8%	4.5%	0.63548	0.95520	0.87709
6 Taxa[3]	87.0%	8.3%	4.7%	0.64020	0.95319	0.86989

[1] Group 1: "Tenerife, La Gomera, Gran Canaria"; Group 2: "El Hierro"; Group 3: "La Palma"; Group 4: "Lanzarote, Fuerteventura"; Group 5: "Morocco"

[2] Group 1: "Tenerife, La Gomera"; Group 2: "Gran Canaria"; Group 3: "El Hierro"; Group 4: "La Palma"; Group 5: "Lanzarote, Fuerteventura, Morocco"

[3] Group 1: "Tenerife, La Gomera"; Group 2: "Gran Canaria"; Group 3: "El Hierro"; Group 4: "La Palma"; Group 5: "Lanzarote, Fuerteventura"; Group 6: "Morocco"

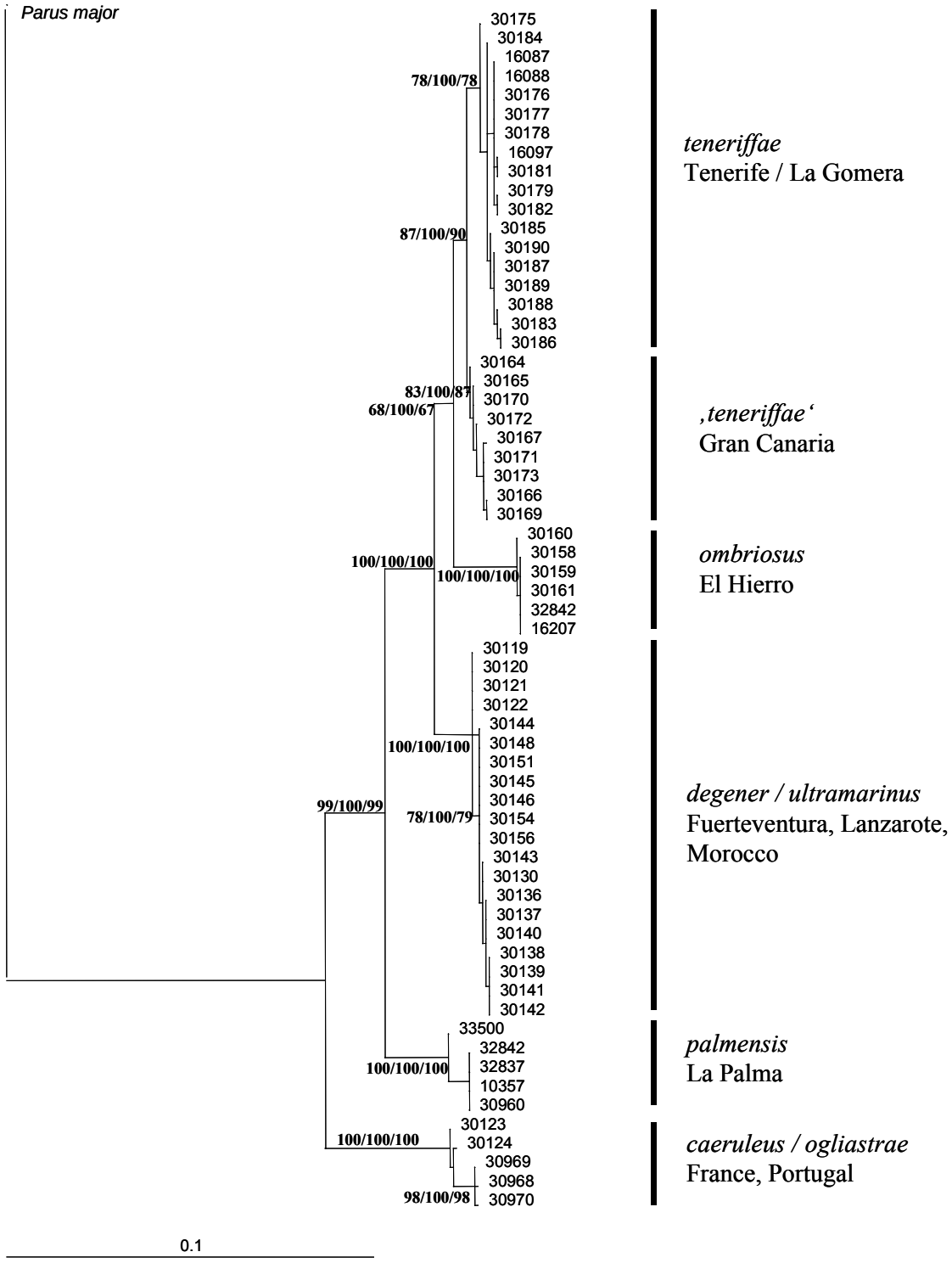

Figure 29 Maximum likelihood phylogram of *Parus caeruleus/teneriffae* taxa based on 1 005 nucleotides of the mitochondrial cytochrome b gene
Numbers indicate bootstrap support (1 000 replicates) of main clades based on Neighbour Joining/Maximum Parsimony/ Maximum Likelihood reconstructions.

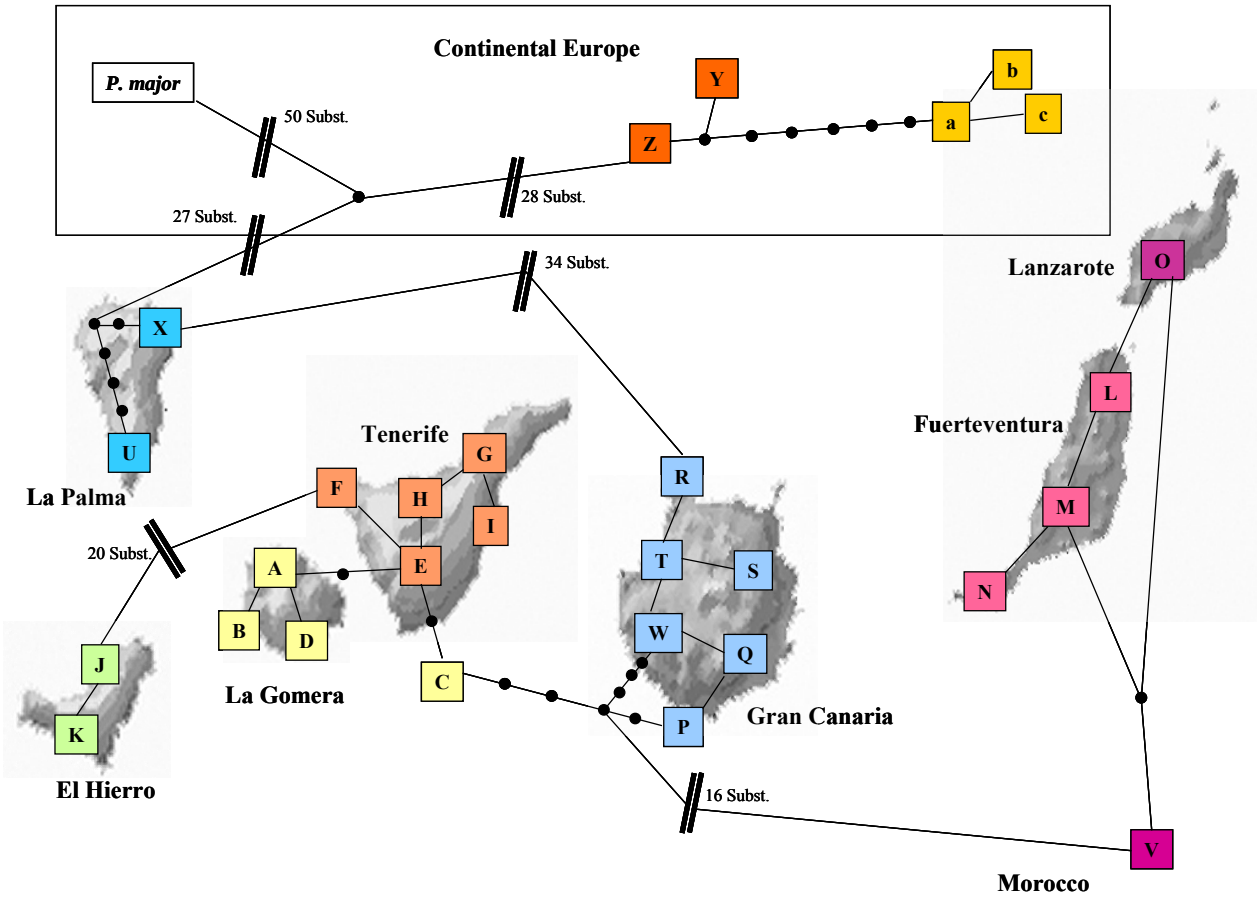

Figure 30 Minimum spanning network of *Parus caeruleus/teneriffae* haplotypes from the Canary Islands and neighbouring areas
Each square represents one haplotype. Black dots mark missing haplotypes and neighbouring haplotypes differ by one nucleotide substitution.

Further evidence comes from the minimum spanning network (Figure 30). The analysis of molecular variance (AMOVA) was tested for different groupings (Table 20) and the classical subspecific division gains lowest support. The highest support was found for the structure comprising the traditional subspecific differentiation (*palmensis*, *ombriosus*, *teneriffae* from Tenerife and La Gomera) plus an additional taxon on Gran Canaria as well as the fusion of birds from Fuerteventura and Lanzarote (classical *degener*) with birds from Morocco (*ultramarinus*). This population structure partitioned 87.7 % of the variation between defined taxa and only 7.8 % and 4.5 % between islands and within islands, respectively. The overall Φ_{ST} was also very high for this scenario (0.95520). Φ_{SC}, describing the variation between islands within defined taxa, was 0.63548 and Φ_{CT} describing the variation between islands was 0.87709.

Pairwise Φ_{ST} values (Table 19) between blue tit populations from the Canary Islands were all high (0.823 – 1.000; mean 0.932 ± 0.080). Lowest values were found between Tenerife – La Gomera (0.637) and Fuerteventura – Lanzarote (0.787). The values between samples from Gran Canaria and Tenerife/La Gomera were also at the lower end of the

range (0.830-0.831). The pairwise Φ_{ST} values increase with increasing distance between populations (Figure 31). These results are further corraborated by the Mantel test, which gave a significant correlation coefficient (r = 0.4690; p < 0.001, coefficient of determination 22.0 %) between pairwise Φ_{ST} values and geographical distances between islands.

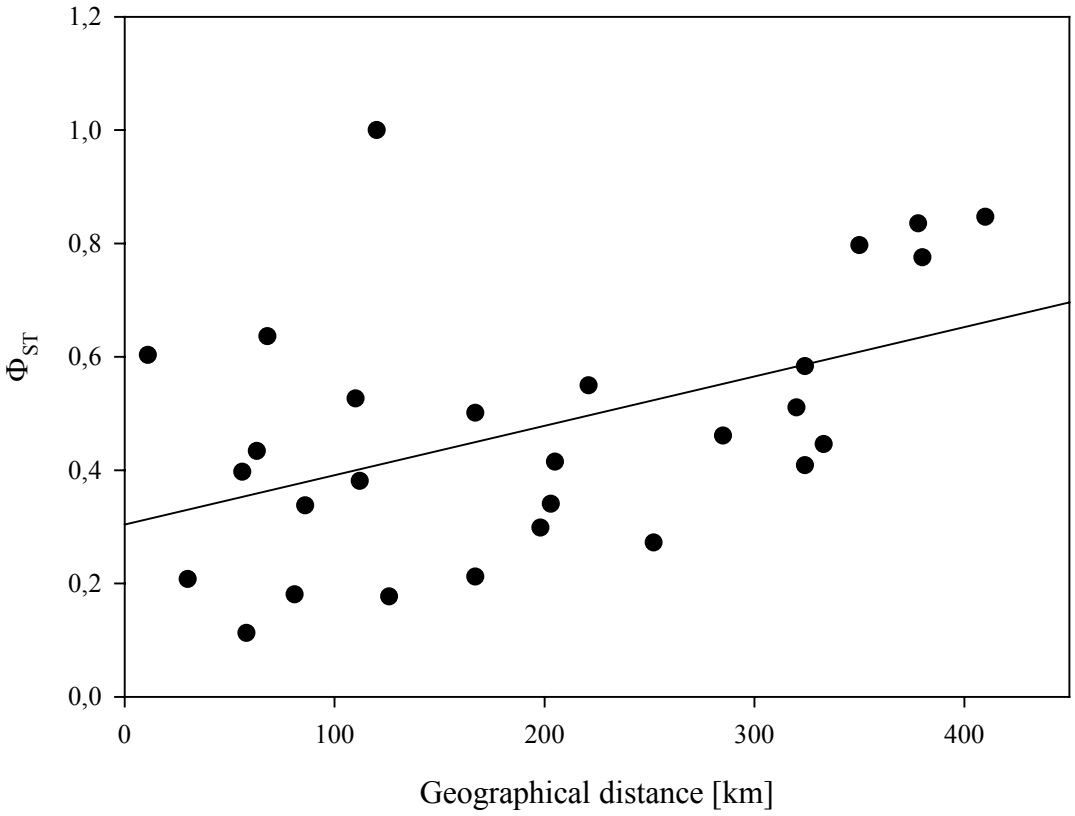

Figure 31 Relationship between pairwise Φ_{ST} values and geographical distance of Blue Tits *P. caeruleus/teneriffae*
Regression curve $y = 8.71 * 10^{-4}x + 0.304$, $R^2 = 0.2201$.

The pairwise mismatch distribution of the combined data set from all Canary Islands displays a very ragged and multimodal curve with peaks at 10, 13, 27, 33, and 41 pairwise nucleotide differences. This is equivalent to divergence times of 280 000–360 000, 360 000–470 000, 750 000–960 000, 920 000–1 200 000 and 1 200 000–1 500 000 years, respectively, (using mitochondrial rate estimates of 2.8–3.6 % per myr, Päckert et al. 2007)[iv]. The mismatch distributions from Tenerife, La Gomera and Fuerteventura were in line with the unimodal distribution expected for population expansion (raggedness statistic r was 0.1582, 0.1867 and 0.1759, respectively). The distributions for La Palma (r = 0.6800), Gran Canaria (r = 0.1088) and El Hierro (r = 0.2222) were more or less

[iv] This analysis is based on the same sequence data (cytochrome b) as presented in this thesis

multimodal, not following the expectations for equilibrium or population growth (Figure 32).

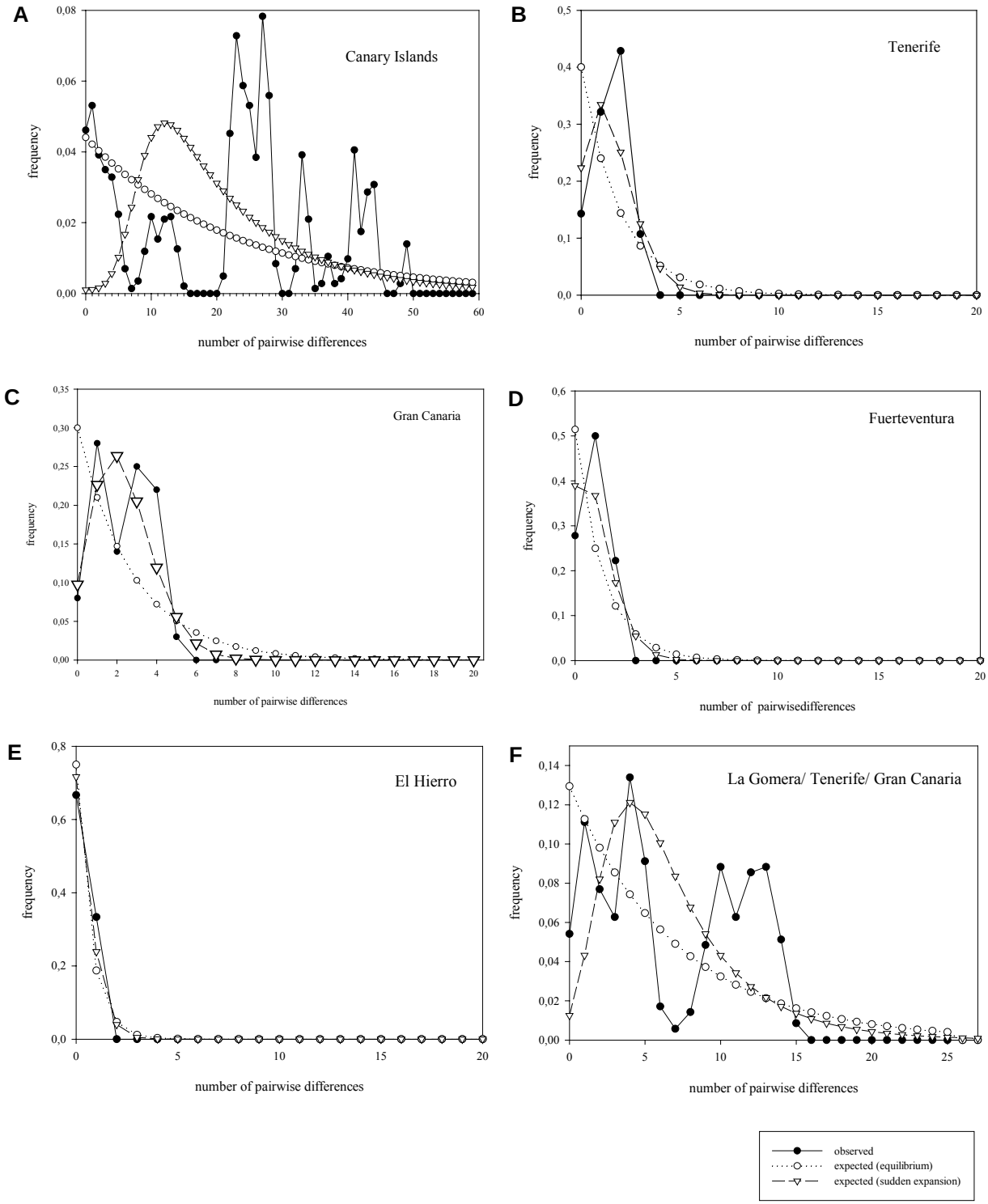

Figure 32 Pairwise mismatch distributions of Canary Island blue tits estimated from mitochondrial cytochrome b sequence data
(A) Combined data for all islands, (B–F) individual islands or island groups with at least six samples.

Morphological measurements

The statistical analysis of variance (ANOVA) of morphological measurements of different populations on the Canary Islands revealed significant differences for a large number of measurements (Table 21). Discriminant function analysis of morphological measurements revealed only weak differentiations. With three measurements included in the discriminant function (length of first secondary, wing length and bill width) only 52.9 % of individuals could be classified correctly. However, significant differences of measurements were found in pairwise comparisons of populations, although these differences were not congruent and displayed large overlap.

Table 21 Morphometric measurements (mean ± s.d.) of *Parus caeruleus* (live birds) from the Canary Islands and the neighbouring mainland
Significance of F from the analysis of variance between island populations (n.s. = not significant, * = p<0.05, **= p<0.01, ***= p<0.001).

	Tenerife n=10	La Gomera n = 8	Gran Canaria n = 12	El Hierro n = 6	La Palma n = 1	Fuerte-ventura n = 19	Lanzarote n = 14	Morocco n = 4	p
Body weight [g]	10.9 ± 0.7	11.2±1.0	11.4±0.8	11.6±0.8	1.50	9.9±0.8	10.7±0.7	9.8±0.4	***
Wing [mm]	63.4 ± 2.4	63.6±2.1	60.7±1.9	61.3±1.9	61.0	61.0±2.3	60.2±2.8	62.8±2.2	**
P9 [mm]	42.0 ± 0.0	39.7±1.1	39.6±1.8	40.5±1.4	38.0	40.19±1.3	40.0±1.5	40.6±0.6	n.s.
P8 [mm]	48.5 ± 2.1	48.0±1.5	46.9±1.9	47.4±1.2	46.5	46.7±1.5	46.6±1.9	48.5±1.7	n.s.
P7 [mm]	50.5 ± 2.1	51.2±1.4	48.9±2.2	49.8±1.5	49.0	48.7±1.6	48.3±1.8	50.5±2.1	*
P6 [mm]	50.0 ± 2.1	52.4±1.1	50.4±2.0	51.3±1.5	51.0	50.1±1.4	49.7±2.0	51.8±2.3	n.s.
P5 [mm]	52.3 ± 1.8	53.1±1.5	51.2±2.0	51.9±1.6	52.0	50.9±1.5	49.6±2.1	52.4±2.1	**
P4 [mm]	51.8 ± 1.8	52.3±1.4	50.8±1.8	51.6±1.6	51.5	49.9±2.3	48.9±1.4	51.0±1.6	**
P3 [mm]	49.3 ± 1.1	50.1±1.4	49.3±1.6	50.3±1.5	50.0	47.9±2.2	47.6±1.4	48.8±1.4	**
P2 [mm]	48.0 ± 1.4	48.2±1.4	48.1±1.4	48.8±1.1	49.0	46.4±1.9	45.5±1.8	47.3±1.4	***
P1 [mm]	47.3 ± 1.8	47.1±1.1	47.1±1.4	48.1±0.9	48.0	45.7±1.4	44.9±1.4	46.5±1.3	***
S1 [mm]	47.8 ± 1.8	48.2±1.1	47.8±1.5	47.4±2.8	48.5	45.5±1.7	45.1±1.2	47.1±2.2	***
Tarsus [mm]	18.1 ± 0.2	18.3±0.8	17.6±0.8	17.8±0.5	18.2	16.6±0.9	16.7±0.4	17.0±1.3	***
NaLoSpi [mm]	7.1 ± 0.1	7.4±0.6	7.3±0.3	7.4±0.6	7.3	6.9±0.3	6.8±0.3	6.7±0.1	**
Bill width [mm]	3.9 ± 0.6	5.3±0.5	4.3±0.4	4.1±0.6	4.3	4.4±0.2	4.5±0.3	4.1±0.3	***
Bill length [mm]	11.6 ± 0.5	12.1±0.5	12.3±0.9	11.9±0.3	11.6	11.9±0.3	11.2±0.5	11.1±0.7	**
Bill height [mm]	3.6 ± 0.1	4.0±0.2	3.7±0.2	3.9±0.4	3.4	4.0±0.2	4.1±0.2	3.7±0.2	***

The most variable parameters found were the bill width and the distance of bill tip to nostril (Figure 33). Birds from Europe differed in many measurements to birds from the Canary Islands and North Africa. Between Canary Island populations larger differences were found between Gran Canaria vs. El Hierro (length of P1: $F = 5.394$, $p = 0.036$; bill length: $F = 6.045$, $p = 0.028$, distance bill tip to nostril: $F = 8.873$, $p = 0.011$) and Fuerteventura/Lanzarote vs. El Hierro (distance bill tip to nostril: $F = 9.503$, $p = 0.004$; bill width: $F = 17.869$, $p < 0.001$; bill depth: $F = 5.647$, $p = 0.024$), while differences between other populations were confined to one or two measurements. Birds of Gran Canaria significantly differed from birds of Tenerife/La Gomera only in bill width ($F = 5.334$; $p = 0.038$).

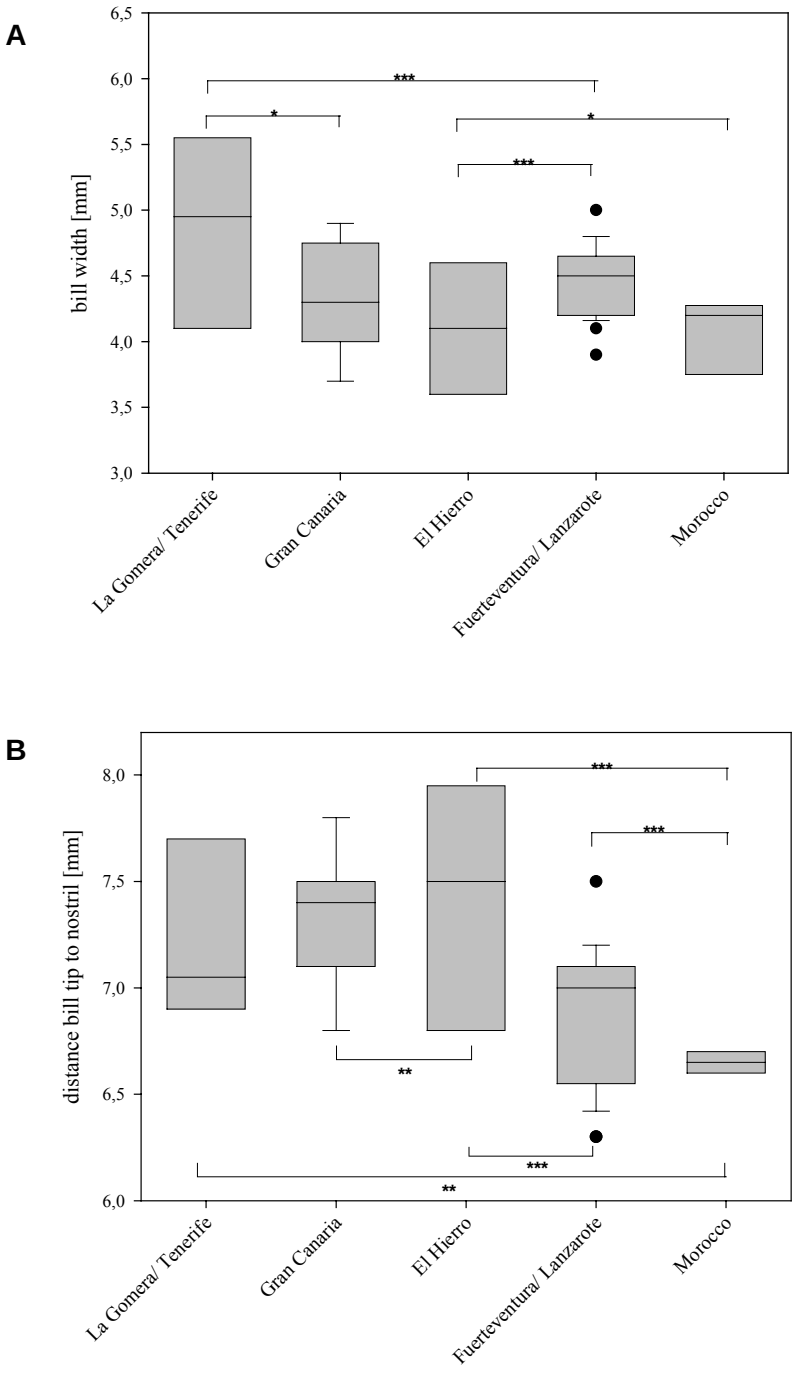

Figure 33 Differences in selected morphometric measurements of *Parus teneriffae* on the Canary Islands illustrated by Box-Whisker-Plot
(A) bill width, (B) distance from bill tip to nostril. Significance of differences is indicated by * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$. Black dots mark outliers.

3.4.4 Discussion

Phylogeography of the blue tit

The present classification of the *Parus teneriffae*–group involves the subspecies *teneriffae* (Gran Canaria, Tenerife, La Gomera), *degener* (Fuerteventura, Lanzarote), *ombriosus* (El Hierro), *palmensis* (La Palma) and *ultramarinus* (Morocco; Martin & Lorenzo 2001, Dickinson 2003, Kvist et al. 2005). Recent investigations on the molecular systematics of Canary Island blue tit taxa on the basis of mitochondrial control region sequence data, as well as morphological and acoustic data from the literature lead to the conclusions that the present classification is valid with the exception of *teneriffae* for which the birds from Gran Canaria represent a distinct subspecies (Kvist et al. 2005).

The mitochondrial cytochrome *b* data also support the validity of the four monophyletic groups suggested by Kvist et al. (2005) including *ombriosus* (El Hierro), *palmensis* (La Palma), *teneriffae* (Tenerife, La Gomera) and an undescribed Taxon on Gran Canaria. Birds from Morocco (*ultramarinus*) did not differ genetically to *degener* from Fuerteventura and Lanzarote. This is supported by as yet unpublished control region data (Kvist 2006). Genetic distances between haplotypes from Gran Canaria and *teneriffae* (La Gomera/ Tenerife) are smaller than distances between other taxa but clearly exceed intraspecific variation of all taxa (0.0–0.5%; Table 19). Consequently, Canary island taxa should be treated as subspecies (Kvist et al. 2005), although genetic distances between island taxa are by all means in the range of species (2.1–4.8 %; Table 19). Nevertheless, there still remain some inconsistencies and ambigous results from different studies and it would be premature to split these taxa without further studies particularly of nuclear data. Nevertheless, the very clear genetic distinction of *P. caeruleus* sensu lato in a northern (*caeruleus*-group) and southern lineage (*teneriffae*-group) was independently found by several authors (Salzburger et al. 2002a, Kvist et al. 2004) and is also supported by this study. The specific recognition of these taxa (*P. caeruleus* sensu strictu and *P. teneriffae*) seems justified. The systematic consequences of my results, considering also other studies (Salzburger et al. 2002a, Kvist et al. 2004, Kvist et al. 2005), comprise the following treatment of allopatric Canary island blue tits until further evidence is available: *P. teneriffae palmensis* (La Palma), *P. t. ombriosus* (El Hierro), *P. t. teneriffae* (Tenerife, La Gomera), *P. t. hedwigii* nov. ssp. (Gran Canaria) and *P. t. ultramarinus* (Fuerteventura, Lanzarote, Morocco). Because of distinct genetic lineages, bioaccoustic evidence and small morphological differences the allopatric blue tit populations from Gran Canaria are considered as a new taxon – *P. t. hedwigii* nov. sp., which is formally described below.

Colonisation of the Canary Islands by the blue tit

Former morphological (Grant 1979a) and genetic studies (Kvist et al. 2005) proposed Tenerife as the origin of island radiations. Currently, an unambiguous conclusion regarding the colonisation pathways does not seem possible, because details differ between studies and sample sizes for all genetic studies (Kvist et al. 2005, this study) were not exhaustive. One reason for the differences in cytochrome b and control region data is a sampling bias with the possibility of inclusion or exclusion of different genetic lineages. Extinction and recolonisation events can have strong influence on the distribution of genetic lineages. It seems likely, that genetic lineages went extinct e.g. due to geological events particularly on the central islands of Tenerife and Gran Canaria, which have a complex geological history (Fuster et al. 1968, Juan et al. 2000). The samples from La Palma form the basis of all Canary Island populations and are clearly separated from other island clades. In Kvist et al. (2005) *palmensis* was even more closely related to *caeruleus* than to other island taxa. Conclusively, a scenario comprising two colonisation events seems possible: (1) an initial colonisation of Tenerife from where the islands of La Gomera, El Hierro and Gran Canaria have been colonised (alternatively Gran Canaria was colonised initially and forms the basis for island radiation) and (2) an independent and more recent colonisation of La Palma, probably by more migratory populations from northern Europe. An immediate connection between La Palma and other islands is not evident from cytochrome b data and La Palma appears to be quite isolated from all other islands supporting independent colonisation events. Independent multiple colonisation events on the Canary Islands have been described for other passerine bird species as well, e.g. *Regulus* (Päckert et al. 2006) or *Fringilla* (Marshall & Baker 1999). There are two lineages containing closely related haplotypes from more than one island (*teneriffae* from Tenerife and La Gomera, *degener*/*ultramarinus* from Fuerteventura, Lanzarote, Morocco), which suggests either ongoing migration, very recent divergences or incomplete lineage sorting.

Description of a new subspecies of blue tit on Gran Canaria

Parus teneriffae hedwigii nov. ssp. Diagnosis: A subspecies of the blue tit, *Parus* [*caeruleus*] *teneriffae*–group, from the islands of Gran Canaria, Canary Islands, Spain, characterised by a dark blue crown encircled by a white supercilium meeting on the front and in the nape, white cheek patch surrounded by black lores, supercilium, throat patch and neck sides, uniformly blue upperparts and tail with a slight greyish cast, uniformly lemon yellow underparts with a diffuse black line in the centre of belly, whitish undertail coverts and a distinctive cluster of mitochondrial cytochrome b (this study) and control region haplotypes Kvist et al. 2005), sequences DQ473999 – DQ474060 (cyt b) and AY538244 – AY538249, AY588286 – AY588288 (ctr. Region; Kvist et al. 2005) GenBank (as opposed

to *P. t. teneriffae*, *P. t. ombriosus*, *P. t. palmensis*, *P. t. degener*, *P. t. ultramarinus*, *P. c. ogliastrae* and *P. c. caeruleus*).

Material: Holotype: G.X.I.c2.ςςς (ZFMK Bonn), male, Gran Canaria, Mogan; Mar. 1908, R. v. Thanner: body length 119.5 mm, wing 64.5 mm, tail 50.0 mm, bill length 11.0 m, bill heigth 3.5 mm.

Paratypes: G.X.I.c2. μμμ (ZFMK Bonn), female, Gran Canaria, Mogan; Mar. 1908, R. v. Thanner: body length 119.0 mm, wing 61.0 mm, tail 45.0 mm, bill length 9.6 mm, bill width 3.5 mm. TFMCVA453 (Museo de Ciencias Naturales, Santa Cruz de Tenerife), male in active moult, Gran Canaria, Saucillo, Galdar; Apr. 2004, Pedro Martín Gómez, body length 109.0 mm, wing length 60.0 mm, tail length 48.0 mm, bill length 10.1 mm, bill depth 4.2 mm.

Additional material: Gran Canaria: three males, four females of the collection of the Zoologisches Forschungsmuseum Koenig (ZFMK), Bonn, Germany.

Comparison material of *P. t. teneriffae* (now restricted to the islands of Tenerife and La Gomera): Tenerife – seven males, five females; La Gomera – 17 males, 15 females; *P. t. ombriosus*: El Hierro – nine males, six females; *P. t. degener*: Fuerteventura – ten males, five females; *P. t. ultramarinus*: Algeria, Tunisia – five males, five females.

General description: outer morphology of *P. t. hedwigii* nov. ssp., especially allover appearance including colouration is very similar to *P. t. teneriffae*. Differences are marginal and include a slightly paler back with a more greyish tinge, a broader black throat patch with more convex lateral edges and a thinner white nape line. Direct comparison is recommended.

Morphology: wing: 60.5–64.5 mm in males (mean = 62.8 ± 2.0 mm, n = 4), 59.0–61.0 mm in females (mean = 60.3 ± 0.8 mm, n = 5); tail: 45.5-52.0 mm in males (mean = 49.1 ± 2.7 mm), 45.0-47.5 mm in females (mean = 45.9 ± 1.1 mm); body length: 110.0-120.5 in males: (mean = 115.5 ± 5.3 mm), 111.0-119.5 mm in females (mean = 114.7 ± 3.6 mm); bill length: 10.1-11.1 mm in males (mean = 10.8 ± 0.5 mm), 9.6-10.5 mm in females (mean = 10.1 ± 0.3 mm); bill depth: 3.4-3.8 mm in males (mean = 3.6 ± 0.2 mm), 3.5-3.7 mm in females (mean = 3.6 ± 0.1 mm). Live birds of *P. t. hedwigii* (both sexes) showed the following measurements (n = 12): wing: 57.0-62.5 mm (mean = 60.7 ± 1.9 mm), P9: 36.0-42.0 mm (mean = 39.69± 1.8 mm), P8: 45.0-49.5 mm (mean = 46.9 ± 1.9 mm), P7: 45.5-52.0 mm (mean = 48.9 ± 2.2 mm), P6: 47.0-52.0 mm (mean = 50.4 ± 2.0 mm), P5: 48.0-53.0 mm (mean = 51.2 ± 2.0 mm), P4: 48.0-53.0 mm (mean = 50.8 ± 1.8 mm), P3: 47.0-51.0 mm (mean = 49.3 ± 1.6 mm), P2: 46.0-50.0 mm (mean = 48.1 ± 1.4 mm), P1: 45.0-48.5 mm (mean = 47.1 ± 1.4 mm), S1: 45.5-50.0 mm (mean = 47.8 ± 1.5 mm), tarsus: 16.5-19.1 mm (mean = 17.6 ± 0.8 mm), bill length: 10.7-13.8 mm (mean = 12.3 ± 0.9 mm), NaLoSpi: 6.8-7.8 mm (mean = 7.3 ± 0.3 mm), bill width: 3.7-4.9 mm (mean = 4.3 ±

0.4 mm), bill depth: 3.4-4.0 mm (mean = 3.7 ± 0.2 mm). For comparative values of *P t. teneriffae* see Table 21.

Vocalisations: The general structure of the territorial song of *P. t. hedwigii* nov. ssp. is similar to other *Parus* taxa and consists of element groups, which again are composed of a different number of elements and are repeated as a group within one strophe (Thielcke 1968, Schottler 1993). The song of *P. t. hedwigii* differs in some aspects from *P. t. teneriffae* of Tenerife/La Gomera. The mean length of song strophes is greater than in all other Canary island Blue tits. This difference is based on the comparatively high number of elements per strophe though mean element length is shorter than in *teneriffae*. Main difference to other taxa of the *teneriffae*-group is the high percentage of element groups within the song repertoire of *P. t. hedwigii* (cf. Schottler 1993).

Ethymology: This clearly characterised and distinct insular subspecies is named in honour of Hedwig Sauer-Gürth, who contributed so much to our laboratory work in this and many other studies at the Institute for Pharmacy and Molecular Biotechnology, University of Heidelberg.

Nomenclature: Lesson (1831) in his description of "*Parus caeruleus teneriffae*" restricted the type locality to the island of Tenerife. In addition, *teneriffae* occurs on La Gomera.

Distribution: On the Canaries the laurel forest has recently been considered as the preferred habitat for the blue tit occupying the western and central islands (Garcia-del-Rey 2003). *Parus teneriffae hedwigii* is restricted to the central island of Gran Canaria, where only 0.5 % of the potential laurel forest area is left untouched (Fernandez 2001). Therefore, on this island, *P. t. hedwigii* is abundant in the pine forest fragments with understory of leguminous shrubs, less common in non-natural forested areas (e.g. *Castanea* sp. and *Eucalyptus* sp.) and scarce in *Euphorbia* scrub and gardens (Sociedad Ornitologica Canaria – Breeding Bird Survey, unpubl. data).

3.5 Phylogenetic differentiation of *Sylvia* species (Aves: Passeriformes) of the Atlantic islands (Macaronesia) based on mitochondrial DNA data and morphometrics

3.5.1 Introduction

According to current knowledge (Martin & Lorenzo 2001), within the passerine genus *Sylvia* three species breed on the Atlantic islands: The Sardinian warbler, *S. melanocephala* (Gmelin, 1789) is distributed around the Mediterranean Sea including coastal areas of southern Europe, the Middle East, North Africa and the Canary Islands (Sibley & Monroe 1990). Four subspecies have been described: *S. m. leucogastra* (Canary Islands), *S. m. melanocephala* (southern Europe, Mediterranean islands, Turkey and North Africa), *S. m. norrisae* (formerly Egypt, extinct) and *S. m. momus* (Syria, Israel, Jordan and Sinai Peninsula; Clements 2000). A recent review on morphological differentiation supports subspecific status of Sardinian warblers, *S. m. leucogastra*, on the Canary Islands and provides evidence for a so far undescribed taxon in northwest Africa (*S. m. valverdei*, Cabot & Urdiales 2005). The blackcap, *S. atricapilla* (Linnaeus, 1758) shows a Palearctic distribution from the Atlantic Islands in the west to the Caucasus in the east with five recognised subspecies: *S. a. gularis* (Cape Verde Islands, Azores), *S. a. heineken* (Spain, Portugal, North Africa, Madeira, Canary Islands), *S. a. atricapilla* (Europe to Siberia), *S. a. pauluccii* (Corsica, Sardinia, Balearics, Italy, Tunisia, Sicilly) and *S. a. dammholzi* (Caucasus, Transcaucasia, Iran; Clements 2000, Shirihai et al. 2001). The spectacled warbler, *S. conspicillata* (Temminck, 1820) has been divided into two subspecies with *S. c. orbitalis* on Madeira, Canary Islands and Cape Verde Islands and *S. c. conspicillata* in the west Mediterranean basin and northwest Africa (Clements 2000, Shirihai et al. 2001).

Sequence data of the mitochondrial cytochrome b gene were used to study the molecular differentiation of *S. melanocephala*, *S. atricapilla* and *S. conspicillata* on the Atlantic islands and to test the phylogenetic relationships of the populations involved, in particular the validity of the distinctiveness of island taxa as proposed by different authors (Clements 2000, Shirihai et al. 2001, Cabot & Urdiales 2005). The genetic data were compared to morphological data from live birds. The analyses focus mainly on *S. melanocephala* and *S. atricapilla*, while *S. conspicillata* was treated only marginally, because of the small sample size currently available. This study is not meant to be exhaustive, but to provide some new insights on evolutionary processes in the genus *Sylvia* and to stimulate further research.

3.5.2 Material and Methods

Samples

The samples for this study were obtained from live birds on the Canary Islands, the Azores, Madeira, Morocco and Portugal in 2001-2005 (Appendix A). Birds were captured with mist-nets, measured, weighed and blood samples were obtained by puncturing the brachial vein. Afterwards, birds were released and blood samples were preserved in storage buffer containing 0.1 M Tris, pH 7.4, 10 % EDTA, 1 % NaF, 0.1 % thymol and frozen at – 20 °C as soon as possible until further processing.

Sequencing

Details of DNA extraction, gene amplification and sequencing reactions are described in chapters 3.1.2 and 3.2.2. The mitochondrial cytochrome b-gene was amplified from total genomic DNA via PCR using the primers L14854 (5'-GGK TCT TTC GCC CTM TC-3'), L14850 (5'-TAC CTG GGK TCT TTC GCC C-3') with mt-Fs-H (H15917; 5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3').

The cycle sequencing reaction (total volume of 10 µl) contained 2 µl of reaction mix (according to the BigDye Terminator Protocol: Applied Biosystems), 10 pmol of the primer L14854 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 obtained sequences could be aligned without difficulties and no stop codons were encountered. The employment of different primers produced overlapping sequences, which gave some additional proof that sequences were correct and of mitochondrial origin.

Phylogenetic Analysis

In addition to sequences collected for this study, further sequences of *Sylvia* species and related taxa deposited at Genbank (AJ534527-AJ534547) were included for initial analysis concerning large-scale phylogeny. *Cettia cetti* (Genbank: AJ004798) was used as an outgroup. The use of different primer combinations produced overlapping sequences with a combined length of up to 1 063 nucleotides from 92 Sardinian warblers, 25 blackcaps and six spectacled warblers. Since the full length could not be obtained for all samples, the following analyses are based on just 917 nucleotides, which were available for the complete data set. Net pairwise genetic p distances and Kimura-2-parameter distances were calculated with MEGA version 2.1 (Kumar et al. 2001). Phylogenetic trees were constructed employing PAUP*4b10 Neighbour-Joining, Maximum Parsimony (Swofford 2001), PHYML, Maximum-Likelihood (Guindon & Gascuel 2003) and MrBayes 2.01 (Huelsenbeck & Ronquist 2001).

Appropriate substitution models for the maximum likelihood calculations was estimated via likelihood ratio test with Modeltest 3.04 (Posada & Crandall 1998). The likelihood settings for different taxonomic units are summarized in Table 22.

Table 22 Likelihood settings of substitution models for different taxonomic units in the genus *Sylvia* estimated via likelihood ratio test (Posada & Crandall 1998).

Taxa	Genus *Sylvia*	*S. melanocephala*	*S. atricapilla*
Model	GTR+I+G	HKY+I+G	HKY+G
Empirical base frequencies:			
pA	0.3223	0.2685	0.2750
pC	0.4146	0.3554	0.3449
pG	0.0819	0.1345	0.1318
pT	0.1811	0.2416	0.2482
Substitution rates:			
$R_{[A-C]}$	0.8861	-	-
$R_{[A-G]}$	12.5255	-	-
$R_{[A-T]}$	1.1606	-	-
$R_{[C-G]}$	0.6306	-	-
$R_{[C-T]}$	12.6109	-	-
$R_{[G-T]}$	1.0000	-	-
Proportion of invariable sites I	0.5852	0.7811	
Gamma shape parameter α	0.9648	0.5099	0.1216
Transition to transversion ratio	-	-	2.2389

Minimum spanning networks were constructed employing TCS 1.13 (Clement et al. 2000). Nucleotide diversity π (Nei 1987), haplotype diversity $\hat{h}$ (Nei 1987), the composite of effective population size (N_e) and mutation rate (μ) as $\theta = 2*N_e*\mu$ (Tajima 1996) and mismatch distributions including raggedness statistics *R*, τ, θ_0 and θ_1 (Harpending 1994) were calculated with DnaSP 4.0 (Rozas et al. 2003). Calculation of divergence time is based on the time measured in mutational events as $\tau = 2ut$ with u as the sum of per-nucleotide mutation rates in the region of DNA under study, which can also be expressed as $u = \mu L$ with the actual mutation rate per nucleotide and generation (μ) and the length of DNA sequence analysed (L), while t measures the time since population size change (Rogers & Harpending 1992, Rogers 1995). Following Pérez-Tris et al. (2004) we use rate estimates for low (0.1 substitutions per site per myr) and high (0.3 s./s./myr) substitution rates, ranging between the maximum rates estimated in comparative studies and the value

estimated in humans (Baker & Marshall 1997, Sigurdardottir et al. 2000). These data are based on the mitochondrial control region and seem appropriate because they can be considered as fastest rates to be expected in the cytochrome b gene. Recent studies showed that substitution rates are not necessarily different between the cytochrome b gene and the control region (Ruokonen & Kvist 2002, Päckert et al. 2006, Päckert et al. 2007). Genetic structure and isolation by distance were evaluated using analysis of molecular variance (AMOVA) and Mantel test employing Arlequin v. 3.0 (Excoffier et al. 1992). Several assumed genetic structures based on taxonomic and geographic groupings were tested.

Morphometrics

All birds captured for DNA sampling were also measured and weighed. The following measurements were taken as described (Svensson 1992): maximum wing length, length of primaries (P) 1-9 and secondary (S) 1, length of tarsus (bent), length of bill tip to distal end of nostril (NaLoSpi), bill width, bill depth and bill length from tip to skull. Measurements were exact to 0.5 mm (wing) and 0.1 mm (leg and bill), respectively. All birds were measured by the same person (CD). 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 pairwise ANOVA for defined groups using SPSS version 10.0.7 (SPSS Inc. 2000). Significance levels were set at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***). To investigate possible morphological differentiation between populations the data were entered into a discriminant function analysis (Wilk's Lambda). Only adult birds not in moult were included. Data were analysed for males and females separately.

3.5.3 Results

The 917 nucleotides in the complete *Sylvia* dataset (21 taxa) showed 297 (32.4 %) variable sites of which 238 (26.0 %) were parsimony informative. Each Macaronesian representative (*S. melanocephala*, *S. atricapilla* and *S. conspicillata*) formed a distinct and well-supported monophyletic clade in the *Sylvia* phylogeny, including island and mainland populations (Figure 34). In the following, the results are detailed for each of the Macaronesian species.

Sardinian warbler (*Sylvia melanocephala*)

The 917 nucleotides sequenced from *S. melanocephala* showed 53 (5.8 %) variable sites of which 42 (4.6 %) were parsimony informative (Appendix E). The net pairwise K2P-distances (and also p-distances) between *melanocephala* populations were very low and ranged from 0.0 to 0.7 % (Table 23). The largest divergences were found between samples from El Hierro and all other areas (0.4–0.7 %). In contrast, high within group distances were evident for Gran Canaria (1.2 %) and eastern Europe (1.1 %).

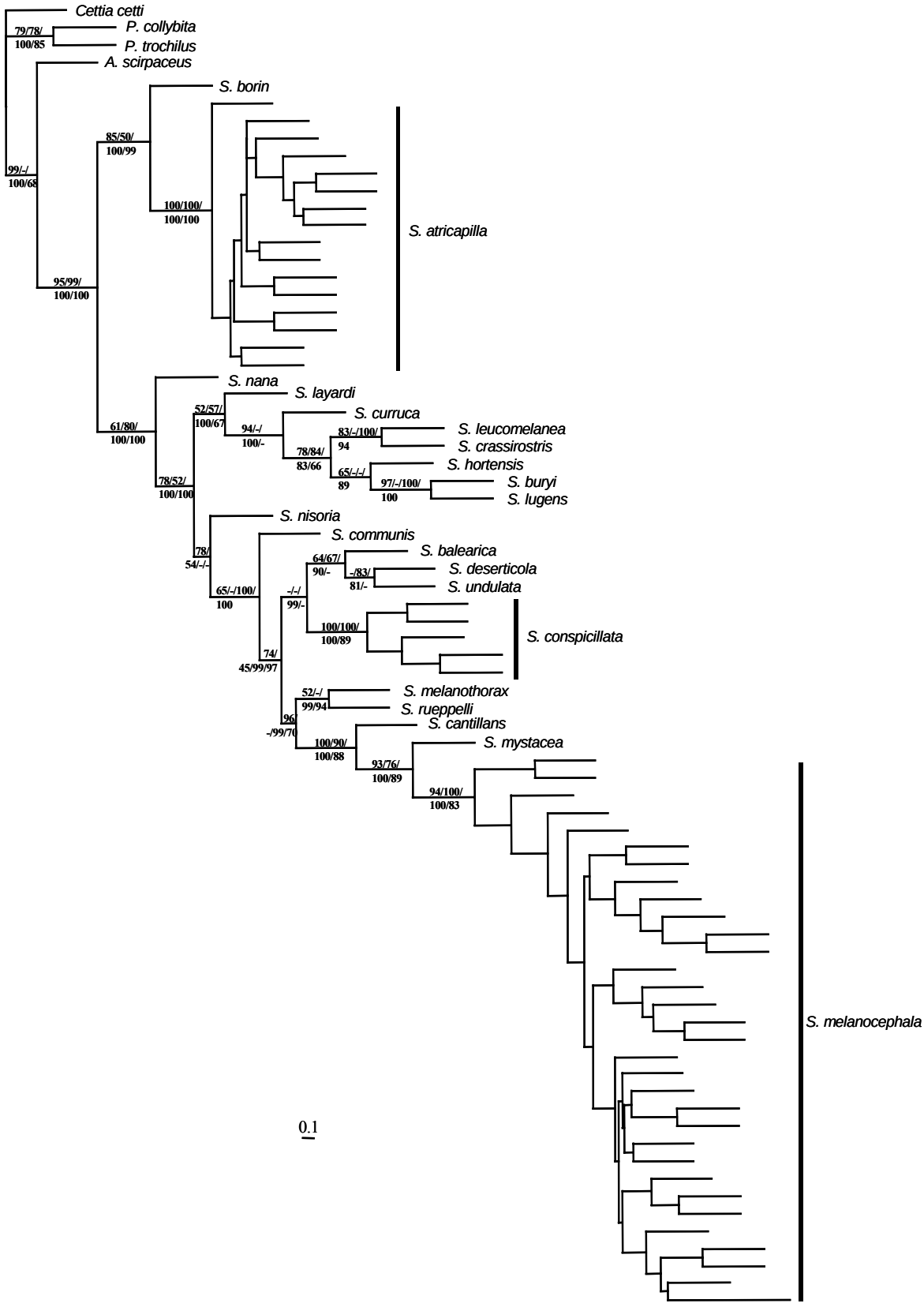

Figure 34 Maximum likelihood phylogram based on mitochondrial cytochrome b sequence data from representatives of the passerine genus *Sylvia*

Numbers indicate bootstrap support and posterior probality values obtained by maximum likelihood/neighbour joining/maximum parsimony/Bayesian inference. '–' means no support or bootstrap values below 50 %.

Table 23 Genetic distances [%] of mitochondrial cytochrome b sequence data for *Sylvia melanocephala* populations and related taxa
Below diagonal net between group mean Kimura-2-Parameter distances, above diagonal genetic p distances and in diagonal within group means (bold).

				S. melanocephala							
		S. cantillans	*S. mystacea*	El Hierro	La Gomera	Gran Canaria	Fuerteventura	Lanzarote	Morocco	W Mediterranean	E Mediterranean
	S. cantillans	–	5.7	6.0	5.8	5.6	6.1	6.0	6.0	5.9	5.4
	S. mystacea	6.1	–	5.6	5.5	5.0	5.7	5.6	5.6	5.5	5.2
S. melanocephala	El Hierro	6.5	6.0	**0.0 0.0**	0.7	0.4	0.6	0.7	0.4	0.6	0.7
	La Gomera	6.2	5.8	0.7	**0.2 0.2**	0.3	0.1	0.3	0.2	0.1	0.3
	Gran Canaria	6.0	5.4	0.4	0.3	**1.2 1.2**	0.1	0.2	0.1	0.2	0.3
	Fuerteventura	6.6	6.1	0.6	0.1	0.2	**0.0 0.0**	0.1	0.0	0.0	0.3
	Lanzarote	6.5	5.9	0.7	0.3	0.2	0.1	**0.0 0.0**	0.2	0.2	0.4
	Morocco	6.4	6.0	0.4	0.2	0.1	0.1	0.2	**0.4 0.4**	0.1	0.2
	W Mediterranean	6.3	5.9	0.6	0.1	0.2	0.0	0.2	0.1	**0.3 0.3**	0.2
	E Mediterranean	5.9	5.5	0.7	0.3	0.3	0.3	0.4	0.2	0.2	**1.1 1.1**

Two haplotypes from Gran Canria (*HM05*, *HM06*) at the basis of the *melanocephala*-clade are clearly distant to all other haplotypes, which explains the high within group variation on this island. The low genetic distances between populations are also reflected by tree topologies obtained through phylogenetic sequence analyses (Figure 35). No tree building algorithm applied did result in a clear pattern that could be correlated with geographic origin of haplotypes and only very few branches gained convincing bootstrap support. Conclusive subgroups within *S. melanocephala* are not evident from cytochrome b data.

The sequences could be assigned to 30 different haplotypes (two additional haplotypes were available from GenBank) and haplotype diversity was 0.818 for the complete data set. Nucleotide diversity for all *melanocephala* sequences was 0.00581. Considering samples from the Canary Islands only, haplotype diversity and nucleotide diversity were lower, h' = 0.710 and π = 0.00574. Highest values on the Canary Islands were found on Gran Canaria (h' = 0.808 and π = 0.01158), but did not surpass values of populations around the Mediterranean regarding haplotype diversity, although nucleotide diversity was

lower there. The same applies for θ values with the highest value on Gran Canaria (θ = 0.00986) opposed to θ = 0.00814 for all Canary Islands and θ = 0.00894 for Mediterranean samples. Selective neutrality for all populations was supported by non-significant Tajima's D values.

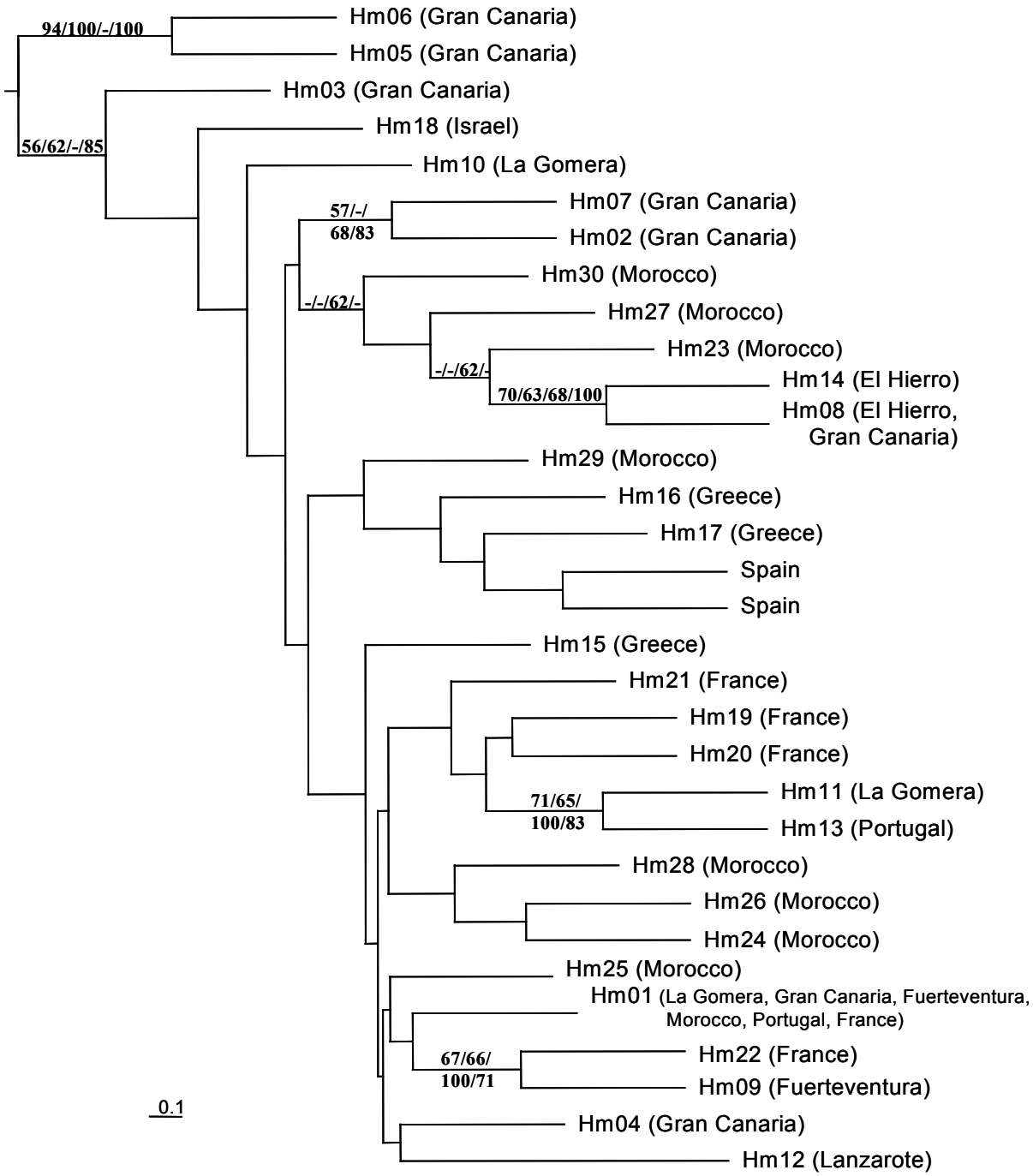

Figure 35 Maximum likelihood phylogram of mitochondrial cytochrome b haplotypes of *Sylvia melanocephala* populations

Trees were rooted with *S. cantillans* (not shown). Numbers indicate bootstrap support and posterior probability values for branching patterns found by maximum likelihood/neighbour joining/maximum parsimony/Bayesian inference. '–' means no support or value below 50 %.

Most haplotypes were confined to one single population although the number of haplotypes varied between populations, which was in part influenced by sample sizes. However, the minimum spanning network does not reveal a clear pattern and geographic lineages are intermixed although most haplotypes were confined to single geographic areas (Figure 36). On the other hand, the widely distributed haplotype *Hm01* was found in all populations except the eastern Mediterranean, which might be artificial due to low sample size.

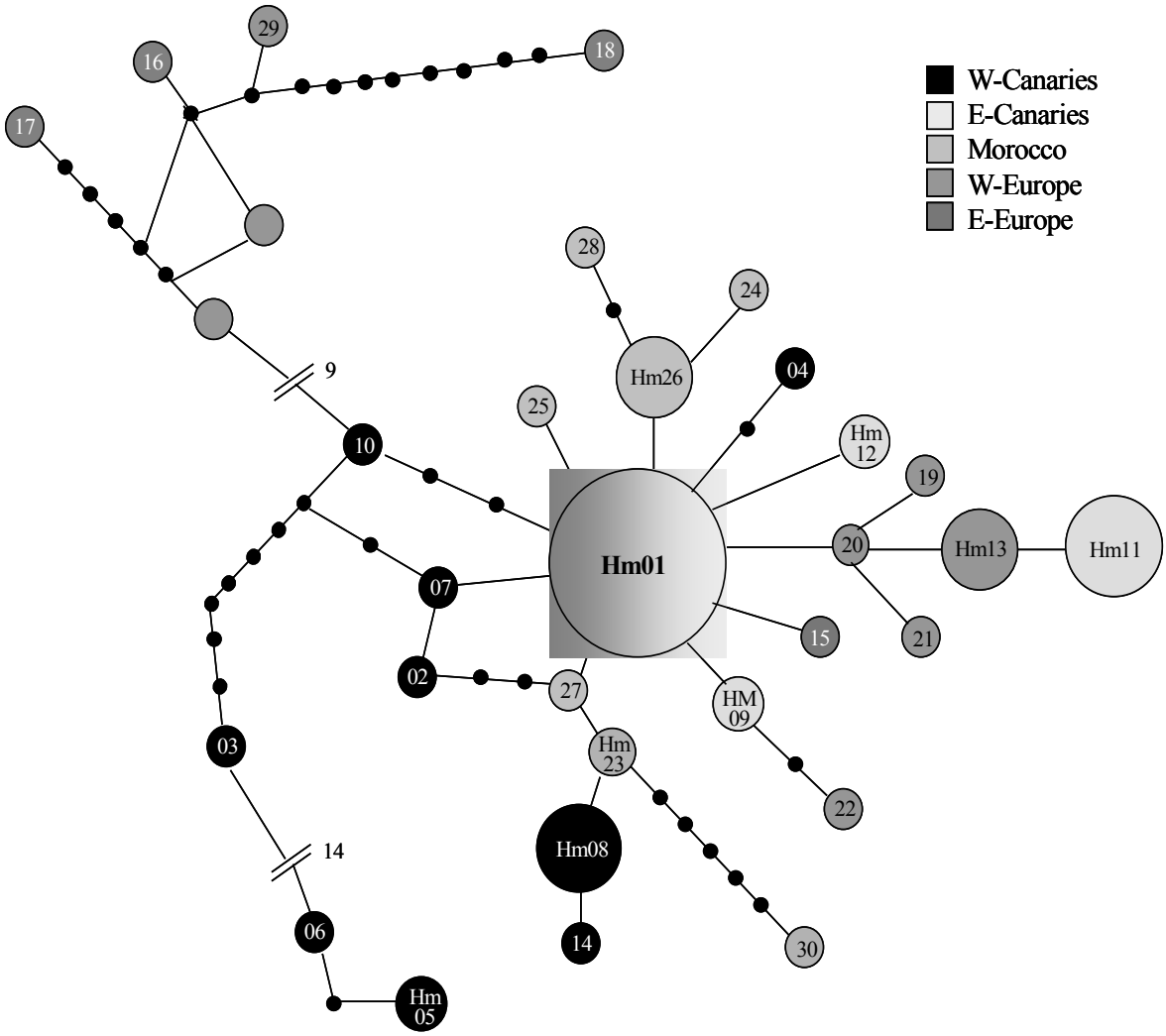

Figure 36 Minimum spanning network of mt cytochrome b haplotypes of *S. melanocephala*
Different shadings indicate geographic distribution of individual haplotypes. Size of circles equals haplotype frequency and numbers designate haplotype (n = 30). Circles without number are related to Genbank sequences.

Differences among populations accounted for a significant 41.1 % of total molecular variance. Hierarchical analyses testing for differences between geographic groupings showed significant differences of the same magnitude among populations within groups.

Only one analysis including two groups with 1) western Canary Islands and 2) eastern Canary Islands, North Africa and continental Europe revealed positive variance components for variation among groups, but still most variation was explained by within population differences. All other tested structures showed slightly negative variance components for among group variation, which could be indicative for absence of genetic structure.

Pairwise Φ_{ST} values (Table 24) between populations from the Canary Islands were rather low but still significant, suggesting restricted gene flow. Highest values were found between El Hierro – Fuerteventura/Lanzarote (0.7506) and El Hierro – La Gomera (0.6461). There is no significant correlation between pairwise Φ_{ST} values and geographical distance of populations (Mantel test, correlation coefficient r = -0.321903; p = 0.776, coefficient of determination 10.4 %).

Table 24 Pairwise Φ_{ST} values of mitochondrial cytochrome b sequence data for *S. melanocephala* populations
Below diagonal pairwise Φ_{st} between populations and above doagonal significance of Φ_{st} values (110 permutations). n.s. = not significant, * $p < 0.05$

	El Hierro	La Gomera	Gran Canaria	Fuerteventura/ Lanzarote	Morocco	W Mediterranean	E Mediterranean
El Hierro	–	*	*	*	*	*	*
La Gomera	0.6461	–	*	*	*	*	*
Gran Canaria	0.4705	0.3020	–	*	*	*	n.s.
Fuerteventura/ Lanzarote	0.7506	0.6286	0.4898	–	*	*	*
Morocco	0.4258	0.2518	0.1439	0.4505	–	*	n.s.
W Mediterranean	0.4909	0.2973	0.1785	0.5078	0.1280	–	n.s.
E Mediterranean	0.5639	0.2744	0.1202	0.5484	0.0581	0.0988	–

The pairwise mismatch distribution of the combined data set from all samples displays a clear peak at three pairwise nucleotide differences followed by some smaller peaks at 6, 14 and 27 pairwise differences (Figure 37). This points to a recent range expansion and some geographic structure. The most recent range expansion can be estimated at 13 000 to 38 000 years BP.

The pairwise analysis of variance (ANOVA) of morphological measurements revealed significant differences for a large number of measurements in males and females (Appendix H). Discriminant function analysis of morphological measurements distinguished between groupings defined by geographic regions although the functions were complex and different for males and females. Even when all 20 measurements were

included in the three discriminant functions it was possible to correctly classify only 86 % and 84 % of males and females, respectively. Measurements showing highest correlations with discriminant functions differed between males and females. High correlations to discriminant functions were evident for bill height, bill width, wing length, foot span and length of P1–P6. Males and females of groups defined according to evolutionary lineages showed clearly separated group means in discriminant analysis, but still there was considerable overlap (Figure 38). Of these groups 86 % of males and 95 % of females could be classified correctly.

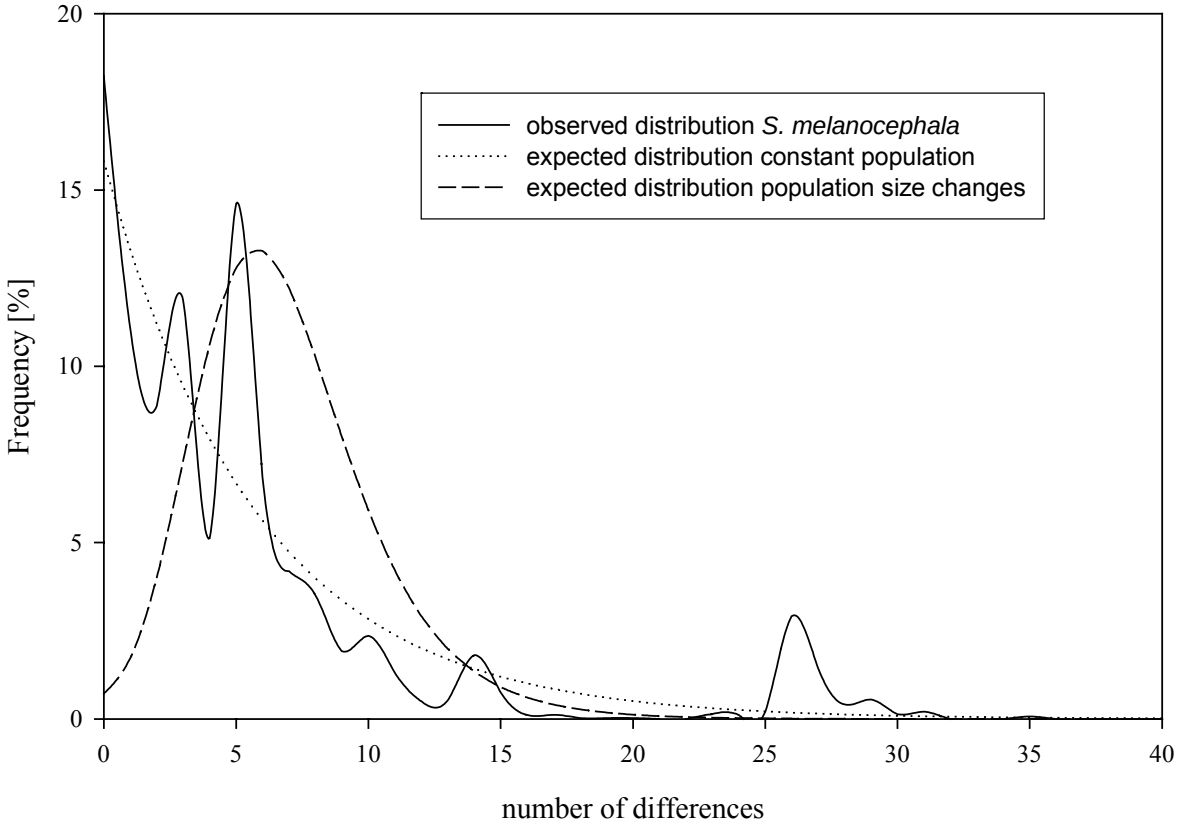

Figure 37 Pairwise mismatch distribution of mitochondrial cytochrome b sequence data of *S. melanocephala*
Observed distributions are compared to expected distributions after models of constant population size and population size changes (Rogers & Harpending 1992).

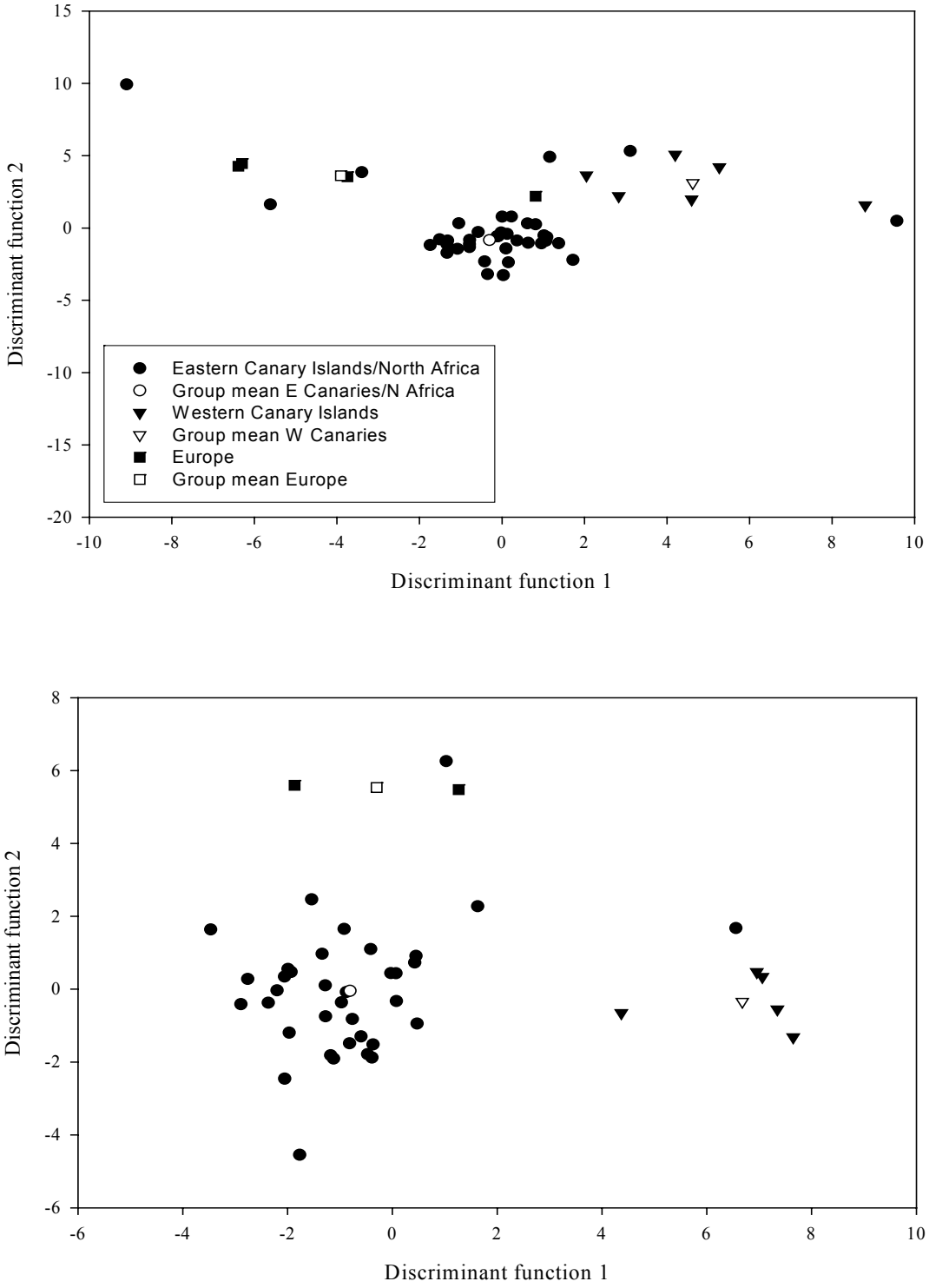

Figure 38 Discriminant function analysis of morphological measurements of *Sylvia melanocephala* from different populations grouped by systematic units
Data were analysed separately for males (above) and females (below).

Blackcap (*Sylvia atricapilla*)

The 917 nucleotides sequenced from *S. atricapilla* showed 13 (1.4 %) variable sites of which eight (0.8 %) were parsimony informative (Appendix F). The net pairwise K2P-distances (and also p distances) between Blackcap populations were very low and ranged from 0.1 to 0.6 % (Table 25). Distances between groups were in the same range as within group distances (0.0 – 0.6 %). The low genetic distances between populations are also reflected by tree topologies obtained through phylogenetic sequence analyses (Figure 39). As in the Sardinian warbler, one haplotype (*Ha10*) from Gran Canaria is located at the base of the blackcap clade. The two haplotypes found on Madeira cluster together opposed to all other haplotypes, which do not show a clear pattern that could be correlated with geographic origin of haplotypes. In general, only very few branches gained convincing bootstrap support.

Table 25 Genetic distances [%] of mitochondrial cytochrome b sequence data for *Sylvia atricapilla* populations and related taxa
Below diagonal net between group mean Kimura-2-Parameter distances, above diagonal genetic p distances and in diagonal within group means (bold).

		S. atricapilla								
	S. borin	Europe	Azores	Madeira	Gran Canaria	Tenerife	La Gomera	El Hierro	La Palma	Morocco
S. borin	–	11.8	11.8	11.8	12.0	11.9	11.9	11.9	11.9	11.9
Europe	13.1	**0.1 0.1**	0.1	0.3	0.3	0.2	0.3	0.4	0.3	0.2
Azores	13.2	0.1	**0.2 0.2**	0.3	0.3	0.2	0.3	0.4	0.3	0.2
Madeira	13.2	0.3	0.3	**0.1 0.1**	0.4	0.4	0.4	0.4	0.4	0.4
Gran Canaria	13.2	0.3	0.3	0.4	**0.6 0.6**	0.4	0.4	0.5	0.5	0.4
Tenerife	13.2	0.2	0.2	0.4	0.4	**0.4 0.4**	0.4	0.6	0.4	0.4
La Gomera	13.2	0.3	0.3	0.4	0.4	0.4	**0.3 0.3**	0.3	0.3	0.3
El Hierro	13.3	0.4	0.4	0.4	0.5	0.6	0.3	**0.0 0.0**	0.3	0.2
La Palma	13.3	0.3	0.3	0.4	0.5	0.4	0.3	0.1	**0.2 0.2**	0.2
Morocco	13.3	0.2	0.2	0.4	0.4	0.4	0.3	0.2	0.2	**0.2 0.2**

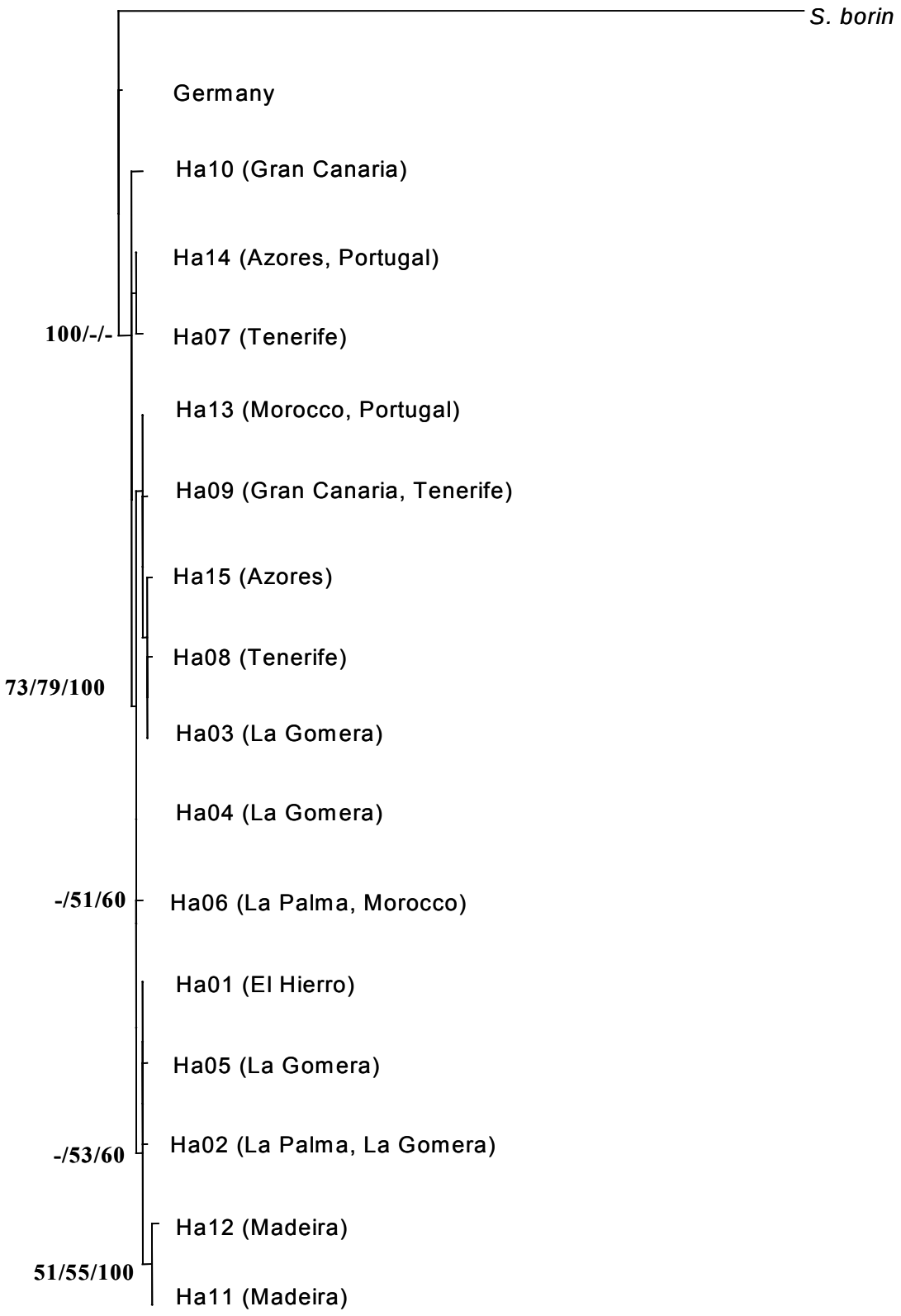

Figure 39 Maximum likelihood phylogram of mitochondrial cytochrome b sequence data of *Sylvia atricapilla* populations
Numbers indicate bootstrap support for branching patterns found by maximum likelihood/neighbour joining/maximum parsimony. '–' means no support or value below 50%.

The sequences could be assigned to 15 different haplotypes and haplotype diversity was 0.954 for the complete data set. Nucleotide diversity for all blackcap sequences was 0.00305. Highest haplotype diversity was found on the Atlantic Islands (h' = 0.971), while respective values were lower for North African and European blackcaps (0.667 and 0.833, respectively). The same pattern is evident for the nucletide diversity, with highest values for Macaronesia (0.00346) and lower values for Europe (0.00127) and North Africa (0.00218). θ was also higher within Macaronesia (0.00406) than in Europe and North Africa (0.00119 and 0.00218, respectively). Within the Atlantic islands the highest h', π and θ were found on the Canary Islands, but sample size was low for other archipelagos. Selective neutrality for all populations was supported by non-significant Tajima's D values.

Most haplotypes were confined to one single geographic population but a clear structure is not shown by the minimum spanning network. Three haplotypes are shared between different biogeographic regions (Figure 40). No haplotype was shared between more than two populations.

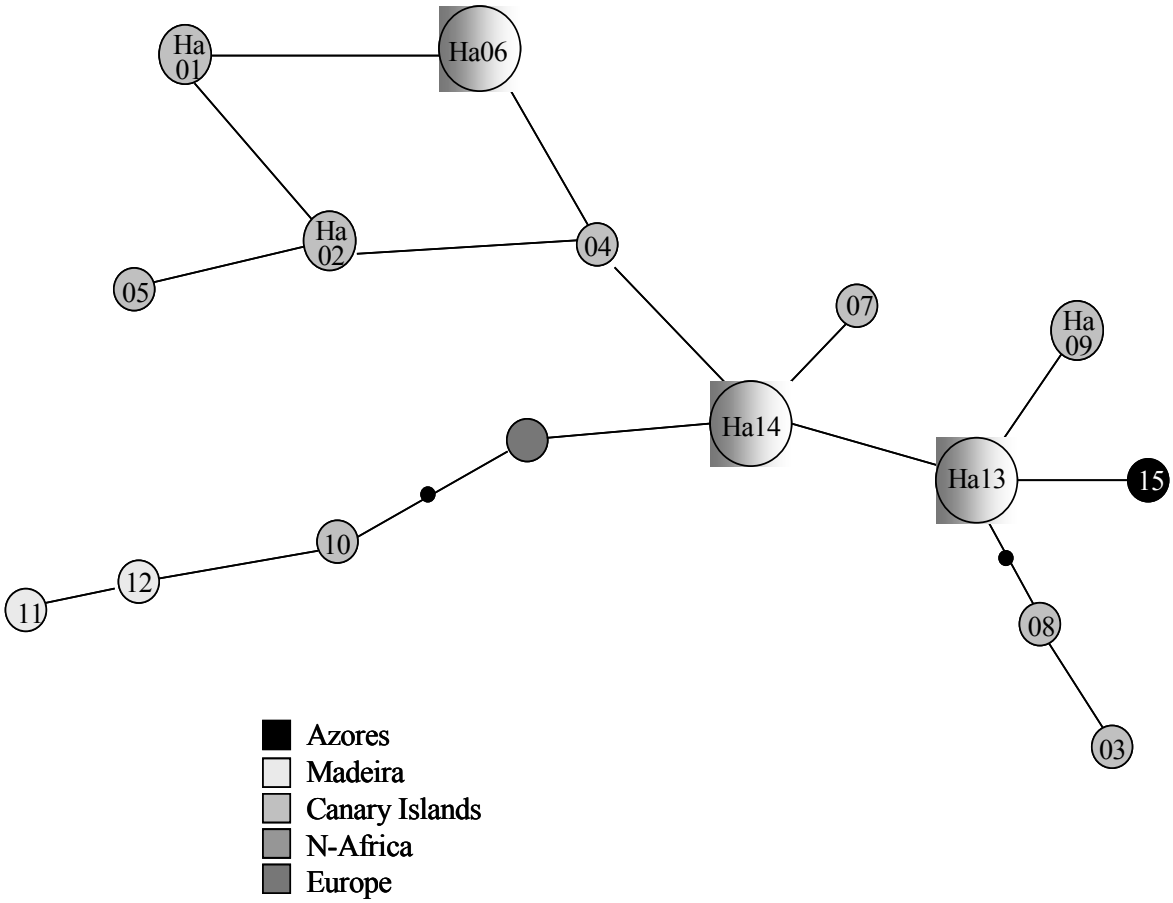

Figure 40 Minimum spanning network of mitochondrial cytochrome b haplotypes of *S. atricapilla*
Different shadings indicate geographic distribution of individual haplotypes. Size of circles equals haplotype frequency and numbers designate haplotype (n = 15).

Differences among populations accounted for a significant 23.1 % of total molecular variance. Hierarchical analyses testing for differences between geographic groupings showed significant differences of the same magnitude among populations within groups. None of these analyses revealed any significant differences between groupings of geographic regions, which always explained less than 5 % of total variance. Most variation is always based on within population differences.

Pairwise Φ_{ST} values (Table 26) between populations were highest for samples from Madeira compared to all other populations (0.1429 – 0.8571). Probably due to generally low sample size significant values were only evident between El Hierro and Europe, and La Palma and Gran Canaria. The correlation between pairwise Φ_{ST} values and geographical distance of populations is not significant but the tendency for Φ_{ST} values to increase with increasing distance is obvious (Mantel test, correlation coefficient r = 0.574733; p = 0.148, coefficient of determination 33.0 %).

Table 26 Pairwise Φ_{st} values of mitochondrial cytochrome b sequence data for *Sylvia atricapilla* populations
Below pairwise Φ_{st} values between populations and above diagonal significance of Φ_{st} values (110 permutations, significance level 0.05). n.s. = not significant, * p < 0.05

	Europe	Azores	Madeira	Gran Canaria	Tenerife	La Gomera	El Hierro	La Palma	Morocco
Europe	–	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Azores	0.1250	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Madeira	0.6866	0.5714	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Gran Canaria	0.0656	0.0282	0.1429	–	n.s.	n.s.	n.s.	*	n.s.
Tenerife	0.1250	-0.0500	0.3551	-0.1413	–	n.s.	n.s.	n.s.	n.s.
La Gomera	0.2994	0.2432	0.2727	0.0820	0.1790	–	n.s.	n.s.	n.s.
El Hierro	0.8800	0.7624	0.8571	0.4444	0.5714	0.1910	–	n.s.	n.s.
La Palma	0.6250	0.5000	0.6827	0.3551	0.4167	0.0968	0.1724	–	n.s.
Morocco	0.2941	0.2105	0.5714	0.1818	0.1724	0.1475	0.3684	0.1818	–

The pairwise mismatch distribution of the combined data set from all samples displays a clearly unimodal curve with one peak at three pairwise nucleotide differences (Figure 41). The mismatch distribution did follow the expectations for a recent range expansion which occurred around 8 000 to 23 000 years BP. The same patter was found for separate analysis of Macaronesian and European/ North African samples.

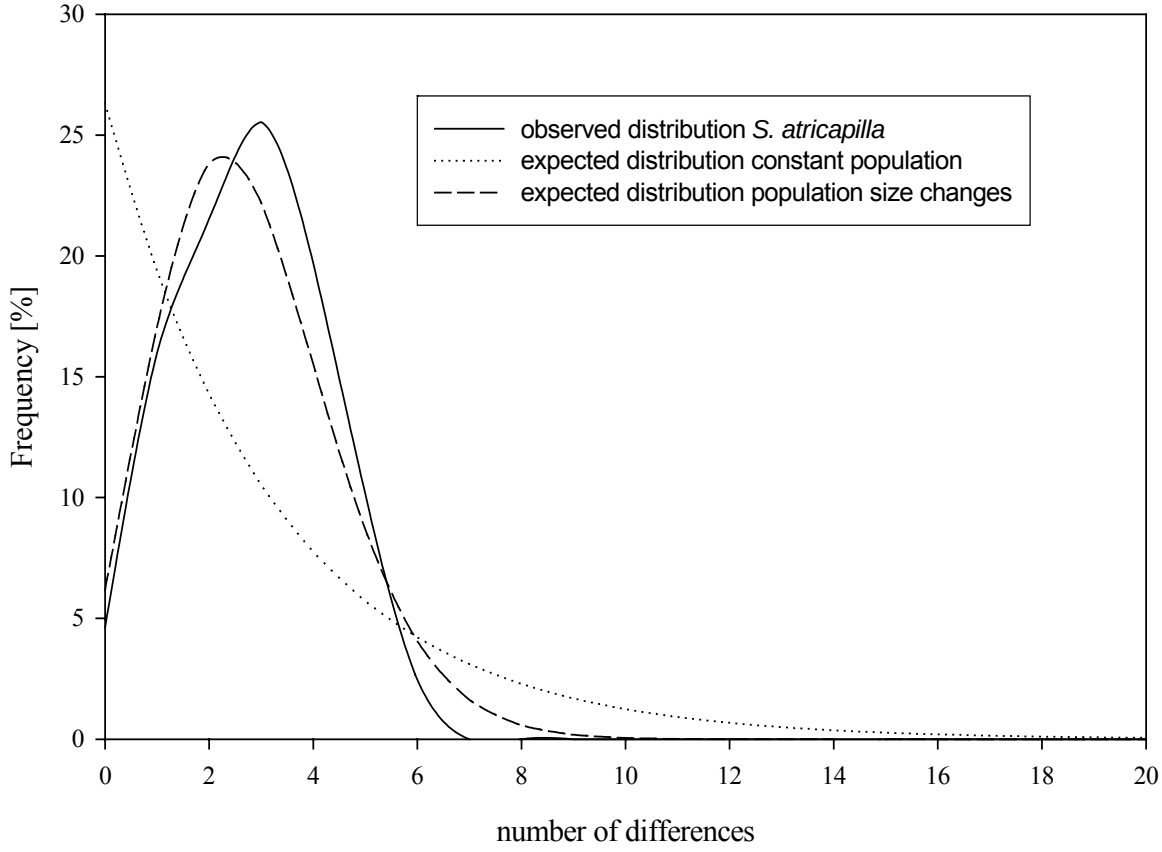

Figure 41 Pairwise mismatch distribution of mitochondrial cytochrome b sequence data of *S. atricapilla*
Observed distributions are compared to expected distributions after models of constant population size and population size changes (Rogers and Harpending 1992).

The pairwise analysis of variance (ANOVA) of morphological measurements revealed significant differences for a large number of measurements in males and females (Appendix I). Discriminant function analysis of morphological measurements distinguished between samples grouped by geographic regions although the functions were complex and differed for males and females. All 20 measurements had to be included in the discriminant functions and only 88 % and 78 % of males and females were classified correctly. Measurements showing highest correlations with discriminant functions differed between males and females. Particularly males from the Canary Islands, Madeira, North Africa and Europe showed large overlap, while males from the Azores were more distinct. Females of these five groups were more similar (Figure 42). High correlations to discriminant functions were evident for lengths of primary feathers. The previously described subspecies *S. a. gularis* (Azores) and *S. a. heineken* (Canary Islands, Madeira, Morocco, Portugal) showed significant differences only for S1, P1–P6 and tarsus length in males, while females showed even more significantly different measurments (S1, P1–P8, tarsus length, wing length, bill length, bill height) and correct classification for this

grouping was better than for the grouping by geographic distribution, with 96 % of males and 90 % of females classified correctly.

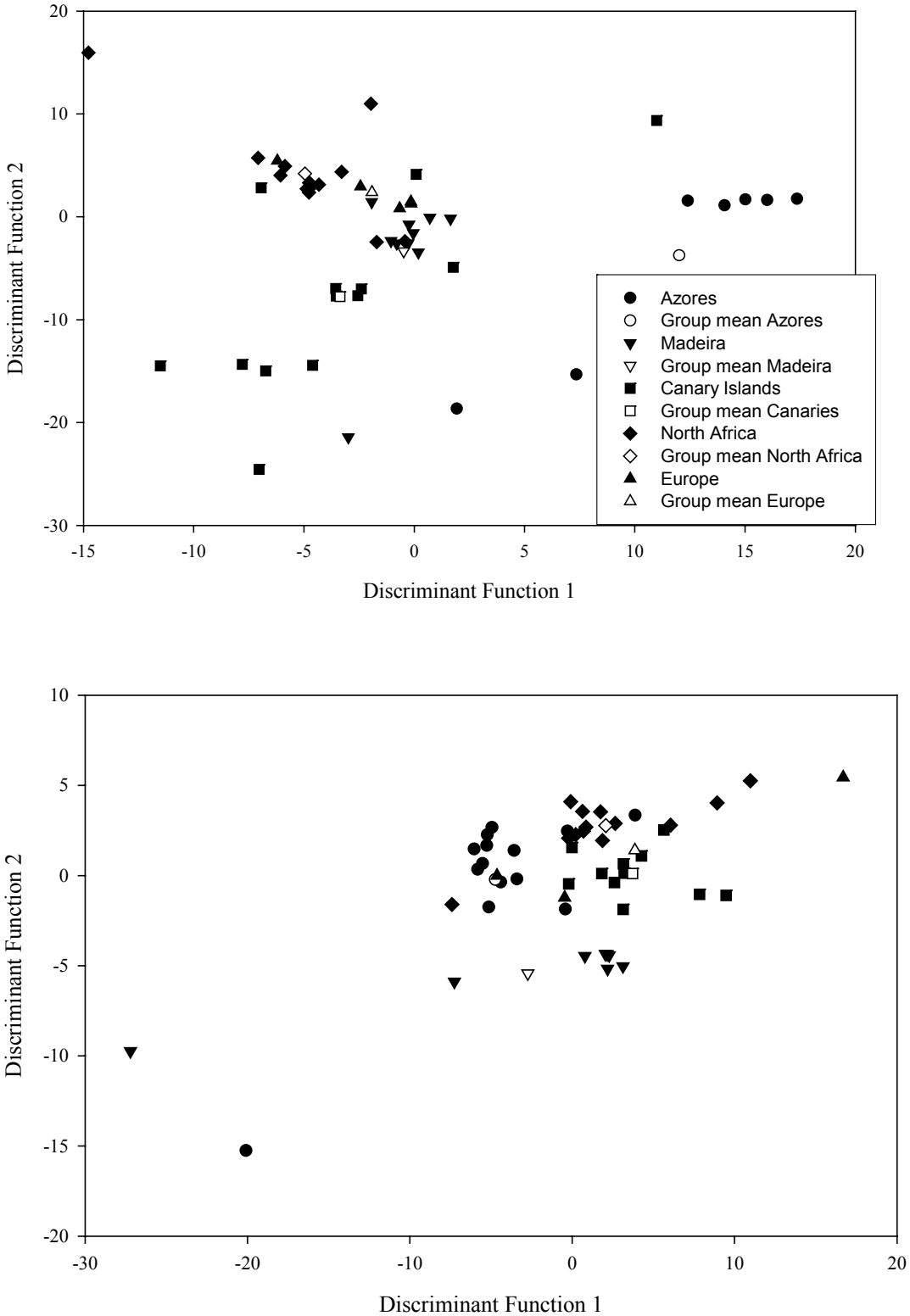

Figure 42 Discriminant function analysis of morphological measurements of *Sylvia atricapilla* populations grouped by geographic regions
Data were analysed separately for males (above) and females (below).

Spectacled warbler (*Sylvia conspicillata*)

The 917 nucleotides in *S. conspicillata* showed 11 (1.2 %) variable sites of which six (0.7 %) were parsimony informative (Appendix G). The net pairwise K2P-distances (and also p distances) between spectacled warbler populations were very low (0.6 %) and in the range of within group distances (0.0 – 0.4 %; Table 27). The sequences could be assigned to five different haplotypes, which were not shared between geographic locations. The two haplotypes found on Lanzarote cluster as a sister group to all other haplotypes from Gran Canaria, Europe and Fuerteventura (Figure 34).

Since most spectacled warblers were in active moult at the time of capture, we do not present and analyse morphological measurements.

Table 27 Genetic distances [%] of mitochondrial cytochrome b sequence data between *Sylvia conspicillata* from the Canary Islands and Europe compared to related taxa
Below diagonal net between group mean Kimura-2-Parameter distances s, above diagonal net p distances and in diagonal within group means (bold).

				S. conspicillata	
	S. undulata	*S. balearica*	*S. deserticola*	Europe	Canary Islands
S. undulata	–	4.2	2.6	6.3	6.3
S. balearica	4.4	–	4.6	7.9	7.9
S. deserticola	2.7	4.9	–	6.5	6.5
Europe	6.7	8.6	7.0	**0.0** **0.0**	0.8
Canary Islands	6.5	8.4	6.8	0.6	**0.4** **0.4**

3.5.4 Discussion

The overall tree topology in the genus *Sylvia* as analysed here is congruent to results of recent molecular studies (Blondel et al. 1996, Shirihai et al. 2001, Böhning-Gaese et al. 2003). Each of the three *Sylvia* species breeding on the Atlantic islands forms a monophyletic group including island and mainland populations and there is no evidence for considerable genetic differentiation within these species.

Phylogeny of Sardinian warbler

The systematic position of Sardinian warblers from the Canary Islands (*S. m. leucogastra*) is still discussed controversly by different authors. On the one hand, morphometric and plumage differences, which clearly exist, are interpreted as clinal and too inconsistent to be

used as discriminating characters (Cramp & Perrins 1992, Shirihai et al. 2001), while the same characters, supported by detailed statistical analyses of morphometrics, provide evidence for subspecific differentiation in this taxon (Cabot & Urdiales 2005). The mitochondrial cytochrome b-sequence data analysed here, show a very low genetic differentiation between birds from the Canary Islands and other areas, which are much lower than distances between other allospecies pairs in the genus *Sylvia*, supporting the results of other studies (Shirihai et al. 2001). Cabot & Urdiales (2005) regard the cytochrome b-gene as inadequate for intraspecific analyses because of low mutation rates. Recent molecular studies have clearly shown, that the cytochrome b-gene is a very useful marker even for intraspecific investigations and reveals similar results as control region data (Dietzen et al. 2003, Kvist et al. 2005, Päckert et al. 2006, Dietzen et al. 2007). Furthermore, the mutation rate is not necessarily lower in the cytochrome b-gene than in, for example, the mitochondrial control region (Ruokonen & Kvist 2002, Päckert et al. 2006, Päckert et al. 2007).

A distinct plumage variation of Sardinian warblers on the Canary Islands has been described by several authors and shows a division into a dark western group (Tenerife, La Palma and probably also La Gomera, El Hierro) and a paler eastern group (Gran Canaria, Fuerteventura, Lanzarote), which is closer in appearance to North African birds (Cramp & Perrins 1992). This has been interpreted as the result of chronologically different invasions to the islands from different source populations, where the darker birds originate from more humid and the paler birds from drier areas (Cramp & Perrins 1992, Cabot & Urdiales 2005). This question cannot be answered by the genetic data of this study, since not all populations are included in the analyses, particularly samples from the *momus/norrisae*–group are missing. However, the tree topologies and haplotype distributions support the theory of repeated colonizations, particularly for Gran Canaria, where a high within group variation was found, which is probably caused by some ancient mitochondrial variants. Interestingly, some birds on Gran Canaria showed very distinct plumage colouration, too (Adam 1983), but a correlation between these birds and distinct haplotypes remains uncertain. The cytochrome b data suggest ongoing differentiation processes (large number of private haplotypes in distinct geographic regions) following either a very recent range expansion and incomplete lineage sorting or are clouded by ongoing gene flow between populations (one very common and widely distributed shared haplotype). The use of several primers producing overlapping sequences and repeated sequencing of each sample excludes sequencing errors, which could also result in haplotypes with restricted frequency and distribution. The results of the mismatch distribution suggest the persistence of some ancient lineages, which cause additional peaks in the mismatch distribution while a recent range expansion certainly has occurred. The restriction of ‘leucogastra’ to birds from the western Canary Islands (El Hierro, La Gomera, Gran Canaria) gains more support from

AMOVA than the inclusion of birds from Fuerteventura and Lanzarote, which are genetically closer to North Africa.
The analysis of morphometrics of live birds did provide similar results as the analysis of museum specimens (Cabot & Urdiales 2005). It must be stressed, that sample size for some groups was very low in our analysis and that different sets of measurements were involved, which might explain differences in the two studies. Furthermore it has to be noted, that these morphological characters (bill measurements, tarsus, wing measurements) are strongly influenced by ecological aspects like migratory behaviour, habitat and diet. Böhning-Gaese et al. (2003) have demonstrated that there is no correlation between phylogeny and ecomorphological traits within the genus *Sylvia*, which in turn means, morphometrics do not necessarily reflect phylogenetic relationships. Instead, the ecomorphological variation, particularly of bill and wing measurements, is mainly explained by migration distance and ecology.
Combining these results, we suggest to treat *S. m. leucogastra* as synonymous to *S. m. melanocephala* because of low genetic differentiation as already proposed (Shirihai et al. 2001). But we encourage future analyses including further samples particularly from the eastern Mediterranean and some Canary Islands to investigate the population genetics and phylogeography in more detail. It would certainly be interesting to learn about the genetic differentiation of the newly described taxon *S. m. valverdei* (Cabot & Urdiales 2005).

Phylogeny of blackcap
Based on colouration and morphological measurements blackcaps on the Atlantic islands have historically been assigned to two subspecies, namely *S. a. gularis* (Azores, Cape Verdes) and *S. a. heineken* (Madeira, Canary Islands; Shirihai et al. 2001). Cytochrome b-sequence data show only very low genetic divergences and all analyses do not provide evidence for considerable genetic differentiation. These findings are in line with results from an exhaustive study on mitochondrial control region data of blackcaps from all over Europe (Pérez-Tris et al. 2004). Pairwise mismatch distributions indicate a very recent range expansion, which tentatively can be dated at 8 000–23 000 years ago. Considering the uncertainties of actual mutation rates, this estimate is very similar to results for control region data of 4 000 to 13 000 years BP (Pérez-Tris et al. 2004). Analysis of molecular variance does not support a population structure within the study area. It has been stated before, that the differences between described subspecies are very subtle (Cramp & Perrins 1992, Shirihai et al. 2001) and mainly can be correlated to migratory and ecological traits (Telleria & Carbonell 1999). This is supported by genetic and morphological findings from this study, which are in line with mitochondrial control region data providing evidence for lacking genetic divergence between populations with different migratory behaviour and related ecomorphological differences (Pérez-Tris et al. 2004). These authors conclude, that

during pleistocene glaciations birds retreated into a glacial refuge in the southern Iberian Peninsula from where the re-colonization of more northern areas took place very recently. Another refuge may have had existed in southeastern Europe and these two refuges are responsible for the migratory divide located in Central Europe. Only populations located on either side of this divide provided evidence for genetic structure in the blackcap, but this still explains only 3.9 % of total variation (Pérez-Tris et al. 2004). The comparison of Atlantic (Madeira, Canary Islands) with continental populations reveals comparable results for control region and cytochrome b data, although variance components are higher for the former (Pérez-Tris et al. 2004). Although our sample size for cytochrome b analyses was not exhaustive, the general results are very similar to control region data. All these findings do not provide evidence for a subspecific differentiation of *S. a. heineken* and *S. a. gularis* (Azores only). Consequently, birds from the Atlantic islands and the southern Iberian Peninsula should be treated as *S. a. heineken* (Jardine, 1830), which has priority over *gularis* (Alexander, 1898). Due to lack of samples the status of *gularis* from the Cape Verdes can not yet be evaluated. The relationship to nominate *atricapilla* remains open, since only one sample from Central Europe was included here. Similarity to other sequences and results from further studies (Telleria & Carbonell 1999, Pérez-Tris et al. 2004) do not point to considerable genetic differentiation between *heineken* and *atricapilla* either.

Phylogeny of spectacled warbler

The sample size for this taxon was very small and allows only some speculative comments. In line with the results for the other two *Sylvia* warblers studied here, the genetic divergence between spectacled warbler sequence data is comparatively low. One haplotype from the Mediterranean basin is not distinctly different to those from the Canary Islands. From these preliminary data there is no evidence for a high degree of genetic divergence between Atlantic and Mediterranean spectacled warblers, and *orbitalis* might be synonymous with *conspicillata*. Certainly more data are required for a comprehensive analysis of this issue.

Conclusions

Particularly the results for Sardinian warbler and blackcap show some common aspects. Both display a lower degree of genetic compared to morphometric differentiation, there is evidence for a recent range expansion and for chronologically different invasions to the Canary Islands. In both species, there are distinct haplotypes and high within group divergences on Gran Canaria, which were probably derived by older colonization events (0.3-3 myr, depending on assumed evolutionary rate estimates, which can be particularly high in small isolated island populations, cf. Päckert et al. 2007). A second colonization

then took place during a recent range expansion, which is comparable for Sardinian warbler and blackcaps (13 000-38 000 and 8 000-23 000 years ago) and coincides with the last post-glacial period. A very distinct haplotype was also found for the blackcap in central Spain (Pérez-Tris et al. 2004) suggesting the preservation of some ancient mitochondrial variants in glacial refuge areas.

The analysis of mitochondrial cytochrome b-sequence data in this study conclusively demonstrates low genetic differentiation in representatives of the genus *Sylvia* on the Atlantic islands – and probably also in general – and supports the ambiguities of ecomorphological and genetic traits. This study should stimulate further research on these taxa with more exhaustive sampling to increase the understanding of factors triggering processes in the evolutionary history of the genus, in particular the role of Pleistocene glaciations.

4 Conclusions and future perspectives

In this study the phylogenetic differentiation and phylogeography of different passerine bird species of the Atlantic islands were investigated with molecular and morphological techniques. The comparison of the results with each other and with results from other island biota allows the deduction of some general aspects about evolution on and colonization of oceanic islands by birds.

4.1 Phylogeograpahic conclusions

Ever since the time of Alfred Russell Wallace (1823-1913) and Charles Darwin (1809-1882), patterns of geographic variation in birds from islands have played an important role in the formulation of evolutionary and biographic theory. In the past, variation among bird populations became an important focus of speciation research as plumage differences were interpreted as an expression of underlying genetic variation, and as evidence of reproductive isolation (Mayr 1963). Today it is possible to build on this foundation using molecular genetic data and new models of population divergence, which allow the identification of barriers of gene flow and historical patterns of genetic diversity that are important factors in the speciation process (Avise 2000). Surveys of intraspecific mitochondrial DNA variation in birds – and other animals – from island archipelagos have clarified historical relationships among populations, patterns of gene flow and colonization, and showed clearly that morphological and molecular divergence are decoupled in many cases (Sato et al. 1999, Böhning-Gaese et al. 2003, Warren et al. 2003, Pérez-Tris et al. 2004, Kvist et al. 2005). Nevertheless, little molecular data have been available so far from birds of the Atlantic islands (Canary Islands, Madeira, Azores), although they provide similar prerequisites as the famous Galapagos Islands, Hawaii or other oceanic archipelagos.

The simplest model of colonization within an archipelago is that of stepping stone colonization, which means younger islands are colonized from neighbouring older islands as is the case in many flightless animals on the Canary Islands (Juan et al. 2000). Also colonizations from younger to older islands can be interpreted as congruent with the stepping stone model if the islands are immediate neighbours to each other. Unfortunately, this simple pattern is complicated by several factors as back colonization, multiple colonizations, recent colonization, within-island differentiation, adaptation and vicariance (Juan et al. 2000). Two methods have been proposed of interpreting phylogenetic tree topologies in terms of colonization sequence on the basis of biogeographical and genetic data, which on the one hand use tree topology and geography or, on the other hand, tree topology and branch lengths (Thorpe et al. 1994a). Both these methods are based on

assumptions (nearest island colonization, founder effects) and violations of these assumptions limit the applicability of the methods (Emerson 2002).

After initial investigations of biogeographic patterns and colonization histories of passerine birds on the Canary Islands based on morphological and bioacoustic evidence (Grant 1979a, 1980), a study of the chaffinch (*Fringilla coelebs*) elucidated the colonization of the Atlantic islands as inferred from molecular data (Marshall & Baker 1999). The initial assumption that the island archipelagos were colonized independently from the geographically closest point on the neighbouring mainland (Grant 1979b) was in parts confirmed by morphology, environmental and ecological factors, but alternative source populations for island colonization existed as well (Grant 1980). The interpretation of molecular evidence favoured a different colonization history than the simple stepping stone model (Marshall & Baker 1999): remnants of an ancestral lineage of common chaffinch still present in Tunesia gave rise to the ancestors of present day continental haplotypes and in rapid succession, this ancestor colonized the Azores, followed by Madeira and the Canary Islands. Furthermore, molecular data suggest subsequent back colonization to Madeira from the Canary Islands. The route within the Canarian archipelago is not obvious, but a likely possibility suggests arrival of chaffinches on the western islands of La Palma and El Hierro (*F. c. palmae*) and subsequent colonization of the central islands La Gomera and Tenerife (*F. c. canariensis*) and finally Gran Canaria (*F. c. canariensis*). Most likely this was a very rapid sequence of events. Conclusively, control region data support one wave of colonization from Europe to the Azores, followed by Madeira and the Canary Islands, and patterns of similarity among Atlantic island chaffinches are due to common colonization history rather than convergent evolution in a common island environment (Marshall & Baker 1999).

Existing molecular phylogenies of other island organisms conform to one of two generalized patterns (Warren et al. 2003): (1) radiations which are contemporary with island formation commonly show a stepwise ‘island colonized as it emerged’ pattern from older to younger islands in the group, as for example in Hawaiian birds (Fleischer et al. 1998); or (2) radiations which post date island formation have shown rapid expansion and speciation, with a short coalescence time within the archipelago, as in Darwin’s finches (Sato et al. 1999, Sato et al. 2001) and birds of the Lesser Antilles (Lovette & Bermingham 1999, Lovette et al. 1999). Some of the investigated passerine bird species of the Atlantic islands (e.g. island canary, chapter 3.2; *Sylvia* warblers, chapter 3.5) show elements of both patterns – periods of rapid expansion, as well as the colonization of new islands from older ones – reflecting the wide range of island ages in the east Atlantic (Figure 43). This intermediate pattern was also found in sunbirds (*Nectarinia* sp.) of western Indian Ocean islands (Warren et al. 2003).

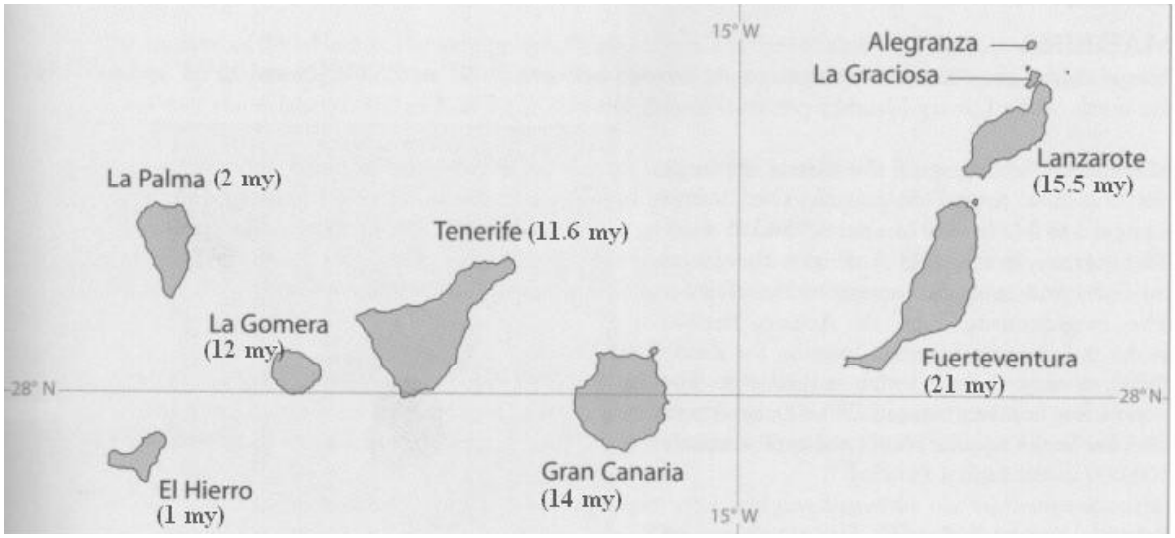

Figure 43 Estimated ages of the Canary Islands

The pattern of colonization history for other species is further complicated by obvious multiple colonizations. The molecular data for the European robin presented in this study support earlier speculations based on morphological characters: after an initial colonization of the central islands Gran Canaria and Tenerife probably from Africa by the ancestor of *E. r. superbus*, followed a second colonization of the western islands (La Palma, El Hierro, La Gomera) from Europe, probably via the Azores and Madeira (Marshall & Baker 1999). The overall pattern in the robin is thus very similar to the colonization of the Atlantic islands by *Fringilla* (Marshall & Baker 1999), for which we also find two separate colonizations of the central islands (Tenerife, Gran Canaria) by ancestors of the blue chaffinch (*F. teydea*) – equivalent to *E. r. superbus* –, followed by a second invasion as detailed above (Marshall & Baker 1999). The intermediate position of *E. r. superbus* (Tenerife) between *E. r. marionae* (Gran Canaria) and *E. r. rubecula* (La Gomera, La Palma, El Hierro) might be explained by introgression of *rubecula* elements into *superbus* through interbreeding after secondary contact between birds from La Gomera and Tenerife following the second colonization. In contrast, the differentiation of the blue chaffinch from a common ancestor with the common chaffinch must have been more advanced and when both came into secondary contact on Tenerife they did not interbreed, i.e. pre-mating barriers were well developed and allowed sympatric co-existence of both species on these islands (Grant 2001).

The colonization of the Atlantic islands by *Regulus* species involves at least three indipendent colonization events which are well supported by molecular data of this study: (1) Madeira was colonized by the firecrest and subsequent isolation lead to the evolution of a distinct (sub)species, the Madeiran firecrest. (2) In a first wave the northeastern Canary Islands of Tenerife and La Gomera were colonized by ancestral goldcrests, followed by a second colonization of the western islands of El Hierro and La Palma. However, the data

do not reject the possibility of monophyly of the Canarian goldcrests. (3) During a more recent wave the Azores have been colonized from Europe and the initial colonization was followed by a radiation after range expansion. The Canarian blue tits have according to present day data reached the Canary Islands in two waves – an initial colonization of either Gran Canaria or Tenerife was followed by dispersal to La Gomera, El Hierro and probably also Fuerteventura, Lanzarote and Morocco. The westernmost island of La Palma was colonized independently by migrants from northern Europe. In the genus *Sylvia* it seems that after a very recent range expansion all Atlantic islands were colonized in a rapid sequence and no genetic differentiation has evolved yet. But in both the blackcap and Sardinian warbler high within island variation on Gran Canaria suggests some ancestral lineages as remains from older colonization events.

One common feature of all Canarian bird species studied here is the fact that island colonization obviously post-dates island formation (Table 28). This is rather surprising because – as flying animals – birds possess exceptional dispersal capabilities. Many passerine bird species, found on the Canary Islands today, have highly migratory relatives in northern Europe. Some disorientated individuals migrating in southwestern direction during autumn migration can be expected to reach the Canary Islands from time to time. In fact, European migrants regularly pass at least through the eastern islands of Fuerteventura and Lanzarote during migration (Martin & Lorenzo 2001, Clarke 2006). How frequent European migrants occur on other islands is largely unclear because the phenotypic separation is not straightforward.

The estimated colonization times are highly dependent on evolutionary rate estimates. Ever since it was suggested that mutations in proteins and their corresponding genes accumulate in a clock-like fashion (Zuckerkandl & Pauling 1965), this hypothesis was used to date events using molecular data. Until very recently, most studies assumed a widely applied universal molecular clock for mitochondrial genes of 2 % sequence divergence per million years, which was based on a calibration in geese (Shields & Wilson 1987). Further molecular clock calibrations remained notably scarce and those studies available suggest some variation in evolutionary rates between and within different bird lineages (Garcia-Moreno 2004, Lovette 2004). Estimated sequence divergences vary between 1.6 – 2 % per million years (Tarr & Fleischer 1993, Krajewski & King 1996, Randi 1996, Fleischer et al. 1998, Nunn & Stanley 1998, Cooper et al. 2001). The crucial factor for rate estimation are the use of adequate calibration points, which are either ancient fossils or palaeogeological events. The correctness of dating these calibration points is essential for reliable rate estimates. Nevertheless, recent critical reviews cast doubt on the validity of the widely applied '2 %-rule' and it can be stated that rates of sequence divergence are not neccessarly equal between different lineages (Ruokonen & Kvist 2002, Garcia-Moreno 2004, Lovette 2004). This is confirmed by calibrations for

mitochondrial cytochrome b data for taxa or at least closely related taxa analysed in this study (Arnaiz-Villena et al. 1999, Päckert et al. 2006, Päckert et al. 2007). There are several factors influencing molecular evolutionary rates, e.g. differences in life histories, generation times, environmental variables, efficiency of DNA-repair systems, population size and population size changes like population bottlenecks and founder effects (Garcia-Moreno 2004). Furthermore, errors associated with the calibration itself have also to be considered, such as phylogenetic uncertainties with fossils and geologic dating errors (Sanderson 1997, Conroy & van Tuinen 2003). Interestingly, recent studies have also rejected the traditional view of faster evolutionary rates in the mitochondrial control region compared to the cytochrome b gene (Ruokonen & Kvist 2002, Päckert et al. 2006, Päckert et al. 2007).

Table 28 Summary of colonization of the Atlantic islands by passerine bird species

Taxon	Number of colonizations	Estimated time of colonization [myr]	Differentiation	Island	Maximum island age [myr]
Erithacus rubecula [a]	2	1.8	*superbus*	Tenerife	12
		2.3	*marionae*	Gran Canaria	14
		0.35	*rubecula*	El Hierro, La Gomera, La Palma	1-12
Serinus canaria [b]	1	1.1	–	Macaronesia	0.3-21
Regulus [c]	(3-)4	4.4	*madeirensis*	Madeira	5
		1.9-2.3	*teneriffae*	Tenerife/La Gomera	11-12
		1.3-1.8	*ellenthaleri*	El Hierro/La Palma	1-2
		0.7	*azoricus/inermis/ sanctaemariae*	Azores	0.3-6
Parus [d, e]	2	1.1-1.5	*palmae*	La Palma	2
		0.6-0.8	*ombriosus*	El Hierro	1
		0.3-0.4	*hedwigii*	Gran Canaria	14
		0.7-1.0	*degener/ultramarinus*	Fuerteventura/ Lanzarote	15-21
Sylvia [a]	≥ 1	0.008-0.04	–	Macaronesia	0.3-21

[a] divergence times calculated after rate estimates of 2 % sequence divergence per my (Shields & Wilson 1987)
[b] divergence times calculated after rate estimates of 0.4 % sequence divergence per my (Arnaiz-Villena et al. 1999)
[c] divergence times calculated after rate estimates of 0.61-0.83 % sequence divergence per my (Päckert et al. 2006)
[d] divergence times calculated after rate estimates of 2.8-3.6 % sequence divergence per my (Päckert et al. 2007)
[e] calculated as divergence from *P. c. teneriffae* on Tenerife

Two reasons seem plausible – also still speculative – explanations for the observed bias between colonization time and island age: (1) the species investigated here inhabitat quiet complex forest habitats and it has to be assumed that the development of these habitats on the islands requires some time. Consequently, forest bird species can not establish breeding populations before the forest habitat has developed. (2) The Atlantic islands have complex volcanic histories (Ancochea et al. 1990, Coello et al. 1992, Ancochea et al. 1999, Juan et al. 2000), which certainly have had big influence on bird and animal populations on the islands, i.e. major volcanic irruptions or land slides could eradicate whole or at least large

parts of island populations (Moya et al. 2004). This could eradicate tracks of successful historical colonization events and today we can only trace the most recent follow-up colonizations. This discrepancy between colonization time of bird species and island age seems not unusual for oceanic archipelagos, e.g. Darwin's finches and Galapagos hawk on the Galapagos Islands (Sato et al. 1999, Bollmer et al. 2006), buff-banded rail, Emerald dove and streaked fantail on Vanuatu (Kirchman & Franklin 2006), orioles on the Lesser Antilles (Lovette et al. 1999), sunbirds on western Indian Ocean islands (Warren et al. 2003) and white-eyes on the Seychelles (Rocamora & Richardson 2003).

With regard to frequent occurrences of European migrants on the Atlantic islands, it seems surprising that island populations still remain genetically distinct from European populations. A recent study on Ryukyu robins from the west Pacific Ryukyu islands has shown that the evolution of sedentary behaviour effectively prevented gene flow between sedentary and migratory populations (Seki et al. 2006). Assortive mating is proposed as an important mechanism to prevent the mixture of sedentary and migratory populations (Bearhop et al. 2005, Seki et al. 2006), which could also apply to Atlantic island bird populations.

In general, Pleistocene glaciations (2 – 0.1 myr BP) are thought to have largely influenced the evolution and biogeography of animal and plant species in Europe. The climatic oscillations had a profound effect on geographic distributions worldwide (Webb & Bartlein 1992). In Europe species retreated from northern areas into three main refuges on the Iberian Peninsula, Italy and the Balkans (Taberlet et al. 1998). The focussed analysis of mitochondrial DNA data supports the importance of Pleistocene effects on speciation processes in birds and other vertebrates (Avise & Walker 1998, Avise et al. 1998). This in mind, it has to be assumed that Pleistocene glaciations have also influenced the colonization and evolution on the Atlantic islands, although more exhaustive data sampling is required to prove this hypothesis. Some authors have even speculated if maybe the Canary Islands also served as glacial refuge from where species might have re-colonized the European mainland (Kvist et al. 2005). At least, this is not contradicted by the genetic data presented here and the results of some species point into this direction as well (e.g. *Erithacus*, *Parus*). However, it is very obvious that evolutionary events within the passerine bird species studied here were dated exclusively in the time range of late Pleistocene glaciations, i.e. 0.008 – 4.4 myr BP (Table 28), as are colonizations of oceanic islands in general.

4.2 Systematic implications

One objective of the present study was the validitation of traditional taxonomic units of the investigated species, which were defined on the basis of morphology and bioacoustics (Volsoe 1951, Vaurie 1959, Bannermann 1963, Bannermann & Bannermann 1965, 1966).

A large advantage of molecular data is that they offer the prospect of reconstructing the phylogeny of a group independently of the morphological features initially used to recognize and systematically organize the group. Instead of interpreting interspecific patterns of morphological variation in order to reconstruct phylogeny, as happened in the past, modern strategies operate in reverse by first reconstructing the phylogeny with molecular data in order to interpret the pattern of morphological or other types of variation (Grant 1998, 2001). This is particularly important for species were morphological variation is rather caused by ecological traits (e.g. migration, diet, habitat) than evolutionary history, as could be shown for several groups of birds (Grant 1998, Böhning-Gaese et al. 2003). Thus, molecular data are helping to resolve the generally recognized problem of assessing the evolutionary and taxonomic status of allopatric populations that are weakly differentiated phenotypically (Mayr 1969, Grant et al. 2000, Grant 2001). Still there are also disadvantages in using molecular data because methods of estimating past evolution rest on uncertain assumptions (Grant 2001). Nevertheless, molecular data have proven particularly useful in discovering hidden differentiation of phenotypically very similar species and they offer a measure of migration rates and gene flow between allopatric populations of a species group, as is often the case on islands (Helbig et al. 1996).

Table 29 Comparison of historical taxonomy based on morphology and bioacoustics to new taxonomic implications of Atlantic island birds inferred from molecular data in this study

Species group	Historical taxonomy	New taxonomic implications
Robins (*Erithacus* sp.)	*E. r. rubecula* (Europe) *E. r. microrhynchus* (Azores, Madeira, El Hierro, La Palma, La Gomera) *E. r. superbus* (Tenerife, Gran Canaria)	*E. r. rubecula* (Europe, Azores, Madeira, El Hierro, La Palma, La Gomera) *E. r. superbus* (Tenerife) *E. r. marionae* nov. ssp. (Gran Canaria)
Crests (*Regulus* sp.)	*R. i. ignicapillus* (Europe) *R. i. madeirensis* (Madeira) *R. r. regulus* (Europe) *R. r. azoricus* (São Miguel) *R. r. inermis* (W Azores) *R. r. sanctaemariae* (S. Maria) *R. r. teneriffae* (Tenerife, La Gomera, El Hierro, La Palma)	*R. ignicapillus* (Europe) *R. madeirensis* (Madeira) *R. r. regulus* (Europe) *R. r. azoricus* (São Miguel) *R. r. inermis* (W Azores) *R. r. sanctaemariae* (S. Maria) *R. r. teneriffae* (Tenerife, La Gomera) *R. r. ellenthalerae* nov. ssp. (El Hierro, La Palma)
Blue tit (*Parus caeruleus*)	*P. c. caeruleus* (N Europe) *P. c. ogliastrae* (Iberia) *P. c. ultramarinus* (N Africa) *P. c. degener* (Fuerteventura, Lanzarote) *P. c. teneriffae* (Gran Canaria, Tenerife, La Gomera) *P. c. ombriosus* (El Hierro) *P. c. palmensis* (La Palma)	*P. c. caeruleus* (N Europe) *P. c. ogliastrae* (Iberia) *P. t. ultramarinus* (N Africa, Fuerteventura, Lanzarote) *P. t. teneriffae* (Tenerife, La Gomera) *P. t. hedwigii* nov. ssp. (Gran Canaria) *P. t. ombriosus* (El Hierro) *P. t. palmensis* (La Palma)
Warblers (*Sylvia* sp.)	*S. m. melanocephala* (Mediterranean) *S. m. leucogastra* (Canary Islands) *S. a. atricapilla* (N Europe) *S. a. heineken* (Iberia, Madeira, Canary Islands) *S. a. gularis* (Azores)	*S. m. melanocephala* (Mediterranean, Canary Islands) *S. a. atricapilla* (N Europe) [*S. a. heineken* (Iberia, Azores, Madeira, Canary Islands)] [a]

[a] *S. a. heineken* might be synonymous to *S. a. atricapilla*

The analysis of molecular data obtained in this study revealed some cases of high genetic differentiation within taxa that had so far been considered monotypic. On the other hand, some morphologically rather distinct taxa showed remarkably low degrees of genetic variation. These findings are discussed in detail in the relevant contributions (chapters 3.1, 3.3, 3.4, 3.5). A summary of taxonomic implications is presented in Table 29. This study disclosed three distinct genetic lineages suggesting a long reproductive isolation and independent evolutionary history from other populations:

1. The robins on the central Canary Islands of Tenerife and Gran Canaria were genetically very different and not even monophyletic. It is recommended to treat the populations on Tenerife (*E. r. superbus*) and Gran Canaria (*E. r. marionae* nov. ssp.) as separate taxa.
2. The goldcrests on the Canary Islands displayed a high degree of genetic, bioacoustic and morphological differentiation. The birds could be assigned into a northeastern group (*R. r. teneriffae* on Tenerife and La Gomera) and a southwestern group (*R. r. ellenthalerae* nov. sp. on El Hierro and La Palma), which do not share common ancestry.
3. The blue tits on the central Canary Islands of Gran Canaria, Tenerife and La Gomera showed a deep split suggesting genetic isolation and reduced gene flow. There is sufficient evidence that birds from Tenerife and La Gomera (*P. t. teneriffae*) have developed independently from the birds on Gran Canaria (*P. t. hedwigii* nov. sp.).

These genetic findings were at least in parts corraborated by morphology and/or bioacoustic data, also these latter differences were often subtle and difficult to detect. In fact, there is a growing body of evidence that genetic structure is frequently masked by phenotypic uniformity (Omland et al. 2000, Kirchman & Franklin 2006). This bias is explained by the principle of convergent evolution, i.e. similar environmental circumstances lead to comparable phenotypes in not closely related taxa (Grant 1998). Consequently, birds of different Canary Islands might be phenotypically very similar due to comparable island environments although they have developed independently over a significant evolutionary timescale. In contrast, there are other species groups displaying large phenotypic differentiation while genetic variation is almost not existent. A very good example for this are the warblers of the genus *Sylvia* on the Atlantic islands, where several subspecies were described in the past on the basis of notable variation in plumage and measurements. But this and other studies (Böhning-Gaese et al. 2003, Pérez-Tris et al. 2004) showed, that genetic variation is very low and both the Sardinian warbler and the blackcap went through a very recent range expansion less than 30 000 years ago. Similar cases of significant morphological differentiation between species that show little if any mitochondrial divergence were found recently, all indicating very recent evolutionary

histories (Seutin et al. 1995, Freeland & Boag 1999, Piertney et al. 2001, Bollmer et al. 2006). The obvious ambiguities between phenotypic and genetic evolution of taxa requires careful interpretation of taxonomic conclusions based solely on morphological variation, which is often caused by environmental factors rather than reflecting evolutionary history. In several investigated taxa of the Atlantic islands we found populations, which were historically described as distinct subspecies but did not show any significant genetic variation. They should be treated as synonymous to respective relatives, e.g. *Erithacus r. microrhynchus* does not differ from *E. r. rubecula*, *Parus t. ultramarinus* does not differ from *P. t. degener* and *Sylvia m. leucogastra* does not differ from *S. m. melanocephala* (Table 29).

This initial study of the molecular evolution of passerine bird species on the Atlantic islands has brought to light many unexpected results. These results have shown that due to uncoupled evolution of phenotype and genotype the actual phylogeny for several taxa differs from traditional opinions. First insights into colonization histories reveal that the colonization of the islands by birds does not follow the simple stepping stone model, but is often complicated by multiple colonizations. Generally, the colonization significantly post-dates island formation and falls in the time of Pleistocene glaciations. In particular the topics of colonization pathways, sequences and timing offer many oportunities for future research, which could provide further surprises once more exhaustive sampling, new genetic markers and analysing methods are available. Furthermore, the comparative analysis of the phylogeography of the Atlantic island avifauna could be completed with the inclusion of further species.

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7 Appendix

Appendix A **Complete list of all samples included in the present study**

Indicated are reference numbers of sample aliquots deposited in the Institute for Pharmacy and Molecular Biotechnology (IPMB), Dept. Biology, University of Heidelberg, Germany), sampling location and Genbank accession number for cytochrome b sequences.

IPMB number	(Sub-) Species	Sampling location	Genbank Acc. Number
10354	*Erithacus rubecula rubecula*	La Palma, Canary Islands	AY286333
10832	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286334
15081	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286335
15082	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286336
15083	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286337
15084	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286338
30103	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286340
30074	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286341
30111	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286342
30108	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286343
30098	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286344
30100	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286345
30101	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286346
30102	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286347
30104	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286348
30105	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286349
30106	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286350
30107	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286351
30110	*Erithacus rubecula superbus*	Tenerife, Canary Islands	AY286352
10831	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286353
30086	*Erithacus rubecula rubecula*	La Palma, Canary Islands	AY286355
30077	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286356
30078	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286357
30080	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286358
30083	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286359
30063	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286360
30068	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286361
30069	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286362
30070	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286363
30087	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286364
30089	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286365
30096	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286366
30095	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286367
30033	*Erithacus rubecula rubecula*	Fuerteventura, Canary Islands	AY286368
30032	*Erithacus rubecula rubecula*	Fuerteventura, Canary Islands	AY286369
30059	*Erithacus rubecula rubecula*	Portugal	AY286370
30060	*Erithacus rubecula rubecula*	Portugal	AY286371
30061	*Erithacus rubecula rubecula*	Portugal	AY286372
30062	*Erithacus rubecula rubecula*	Portugal	AY286373
30064	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286374
30065	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286375
30066	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286376
30067	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286377
30071	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286378
30072	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286379
30073	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286380
30075	*Erithacus rubecula rubecula*	El Hierro, Canary Islands	AY286381
30079	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286382
30081	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286383
30082	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286384
30084	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286385
30076	*Erithacus rubecula rubecula*	La Gomera, Canary Islands	AY286386

IPMB number	(Sub-) Species	Sampling location	Genbank Acc. Number
30088	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286387
30090	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286388
30091	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286389
30092	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286390
30093	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286391
30094	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286392
30097	*Erithacus rubecula marionae*	Gran Canaria, Canary Islands	AY286393
24648	*Serinus canaria*	Pico, Azoren	AY914108
24649	*Serinus canaria*	Pico, Azoren	AY914109
24650	*Serinus canaria*	Pico, Azoren	AY914110
24651	*Serinus canaria*	Pico, Azoren	AY914111
24652	*Serinus canaria*	Pico, Azoren	AY914112
24653	*Serinus canaria*	Pico, Azoren	AY914113
24654	*Serinus canaria*	Pico, Azoren	AY914114
24655	*Serinus canaria*	Pico, Azoren	AY914115
24656	*Serinus canaria*	Pico, Azoren	AY914116
24657	*Serinus canaria*	Pico, Azoren	AY914117
24658	*Serinus canaria*	Pico, Azoren	AY914118
24659	*Serinus canaria*	Pico, Azoren	AY914119
24661	*Serinus canaria*	Pico, Azoren	AY914120
24662	*Serinus canaria*	Pico, Azoren	AY914121
24704	*Serinus canaria*	Ilheu Chão, Madeira	AY914122
24708	*Serinus canaria*	Ilheu Chão, Madeira	AY914123
24736	*Serinus canaria*	Ilheu Chão, Madeira	AY914124
24728	*Serinus canaria*	Ilheu Chão, Madeira	AY914125
24758	*Serinus canaria*	Madeira	AY914126
24760	*Serinus canaria*	Madeira	AY914127
24762	*Serinus canaria*	Madeira	AY914128
24763	*Serinus canaria*	Madeira	AY914129
24765	*Serinus canaria*	Madeira	AY914130
24766	*Serinus canaria*	Madeira	AY914131
24767	*Serinus canaria*	Madeira	AY914132
24768	*Serinus canaria*	Madeira	AY914133
24769	*Serinus canaria*	Madeira	AY914134
24784	*Serinus canaria*	Madeira	AY914135
24802	*Serinus canaria*	Madeira	AY914136
24705	*Serinus canaria*	Ilheu Chão, Madeira	AY914137
24706	*Serinus canaria*	Ilheu Chão, Madeira	AY914138
24707	*Serinus canaria*	Ilheu Chão, Madeira	AY914139
24714	*Serinus canaria*	Ilheu Chão, Madeira	AY914140
24715	*Serinus canaria*	Ilheu Chão, Madeira	AY914141
24716	*Serinus canaria*	Ilheu Chão, Madeira	AY914142
24717	*Serinus canaria*	Ilheu Chão, Madeira	AY914143
24718	*Serinus canaria*	Ilheu Chão, Madeira	AY914144
24720	*Serinus canaria*	Ilheu Chão, Madeira	AY914145
24721	*Serinus canaria*	Ilheu Chão, Madeira	AY914146
24722	*Serinus canaria*	Ilheu Chão, Madeira	AY914147
24723	*Serinus canaria*	Ilheu Chão, Madeira	AY914148
24724	*Serinus canaria*	Ilheu Chão, Madeira	AY914149
24727	*Serinus canaria*	Ilheu Chão, Madeira	AY914150
24729	*Serinus canaria*	Ilheu Chão, Madeira	AY914151
24730	*Serinus canaria*	Ilheu Chão, Madeira	AY914152
24731	*Serinus canaria*	Ilheu Chão, Madeira	AY914153
24732	*Serinus canaria*	Ilheu Chão, Madeira	AY914154
24734	*Serinus canaria*	Ilheu Chão, Madeira	AY914155
24737	*Serinus canaria*	Ilheu Chão, Madeira	AY914156
24738	*Serinus canaria*	Ilheu Chão, Madeira	AY914157
24739	*Serinus canaria*	Ilheu Chão, Madeira	AY914158
24740	*Serinus canaria*	Ilheu Chão, Madeira	AY914159

IPMB number	(Sub-) Species	Sampling location	Genbank Acc. Number
24741	*Serinus canaria*	Ilheu Chão, Madeira	AY914160
24742	*Serinus canaria*	Ilheu Chão, Madeira	AY914161
24743	*Serinus canaria*	Ilheu Chão, Madeira	AY914162
1590	*Regulus ignicapillus*		AY894885
3371	*Regulus ignicapillus*	Jan Festo, France	AY894887
1582	*Regulus ignicapillus*		AY894888
3391	*Regulus ignicapillus*	Jan Festo, France	AY894886
30342	*Regulus madeirensis*	Madeira	AY894865
30339	*Regulus madeirensis*	Madeira	AY894866
30350	*Regulus madeirensis*	Madeira	AY894867
30351	*Regulus madeirensis*	Madeira	AY894868
30344	*Regulus madeirensis*	Madeira	AY894869
30352	*Regulus madeirensis*	Madeira	AY894870
30345	*Regulus madeirensis*	Madeira	AY894871
30353	*Regulus madeirensis*	Madeira	AY894872
30346	*Regulus madeirensis*	Madeira	AY894873
30354	*Regulus madeirensis*	Madeira	AY894874
30347	*Regulus madeirensis*	Madeira	AY894875
30348	*Regulus madeirensis*	Madeira	AY894876
30349	*Regulus madeirensis*	Madeira	AY894877
30343	*Regulus madeirensis*	Madeira	AY894878
22438	*Regulus regulus*	Kornberg, Germany	AY894879
966	*Regulus regulus*	Braunschweig, Germany	AY894880
10252	*Regulus regulus*	Jan Festo, France	AY894881
1836	*Regulus regulus*	Chur, Switzerland	AY894882
1571	*Regulus regulus*	Innsbruck, Austria	AY894883
1576	*Regulus regulus*		AY894884
30356	*Regulus regulus azoricus*	Terceira, Azores	AY894859
30357	*Regulus regulus azoricus*	Terceira, Azores	AY894860
30355	*Regulus regulus azoricus*	Terceira, Azores	AY894861
30358	*Regulus regulus azoricus*	Terceira, Azores	AY894862
30360	*Regulus regulus azoricus*	Terceira, Azores	AY894863
30359	*Regulus regulus inermis*	São Miguel, Azores	AY894864
30373	*Regulus teneriffae ellenthalerae*	La Palma, Canary Islands	AY894837
30374	*Regulus teneriffae ellenthalerae*	La Palma, Canary Islands	AY894838
30361	*Regulus teneriffae ellenthalerae*	El Hierro, Canary Islands	AY894839
30362	*Regulus teneriffae ellenthalerae*	El Hierro, Canary Islands	AY894840
30367	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894841
30364	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894842
30363	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894843
30365	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894844
30366	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894845
30375	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894846
30376	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894847
30377	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894848
30378	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894849
30368	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894850
30369	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894851
30370	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894852
30383	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894853
30382	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894854
30380	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894855
30372	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894856
30379	*Regulus teneriffae teneriffae*	Tenerife, Canary Islands	AY894857
30371	*Regulus teneriffae teneriffae*	La Gomera, Canary Islands	AY894858
16087	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ473999
16088	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474000
16097	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474001
32842	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474020

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16207	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474021
30969	*Parus caeruleus caeruleus*	France	DQ474041
30968	*Parus caeruleus caeruleus*	France	DQ474042
30970	*Parus caeruleus caeruleus*	France	DQ474043
32836	*Parus teneriffae palmensis*	La Palma, Canary Islands	DQ474044
32837	*Parus teneriffae palmensis*	La Palma, Canary Islands	DQ474045
10357	*Parus teneriffae palmensis*	La Palma, Canary Islands	DQ474046
30960	*Parus teneriffae palmensis*	La Palma, Canary Islands	DQ474047
33500	*Parus teneriffae palmensis*	La Palma, Canary Islands	DQ474061
30175	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474002
30176	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474003
30177	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474004
30178	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474005
30179	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474006
30181	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474007
30182	*Parus teneriffae teneriffae*	La Gomera, Canary Islands	DQ474008
30184	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474009
30185	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474010
30188	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474011
30190	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474012
30183	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474013
30186	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474014
30187	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474015
30189	*Parus teneriffae teneriffae*	Tenerife, Canary Islands	DQ474016
30158	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474017
30159	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474018
30161	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474019
30160	*Parus teneriffae ombriosus*	El Hierro, Canary Islands	DQ474022
30143	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474023
30130	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474024
30136	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474025
30137	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474026
30138	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474027
30139	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474028
30140	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474029
30141	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474030
30142	*Parus teneriffae degener*	Fuerteventura, Canary Islands	DQ474031
30144	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474032
30148	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474033
30151	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474034
30145	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474035
30146	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474036
30154	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474037
30156	*Parus teneriffae degener*	Lanzarote, Canary Islands	DQ474038
30123	*Parus caeruleus ogliastrae*	Portugal	DQ474039
30124	*Parus caeruleus ogliastrae*	Portugal	DQ474040
30119	*Parus teneriffae ultramarinus*	Morocco	DQ474048
30120	*Parus teneriffae ultramarinus*	Morocco	DQ474049
30121	*Parus teneriffae ultramarinus*	Morocco	DQ474050
30122	*Parus teneriffae ultramarinus*	Morocco	DQ474051
30164	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474052
30165	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474053
30166	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474054
30167	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474055
30169	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474056
30170	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474057
30171	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474058
30172	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474059
30173	*Parus teneriffae hedwigii*	Gran Canaria, Canary Islands	DQ474060

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30478	*Sylvia atricapilla*	Morocco	EF446839
30483	*Sylvia atricapilla*	Morocco	EF446839
30542	*Sylvia atricapilla heineken*	La Palma, Canary Islands	EF446839
30544	*Sylvia atricapilla heineken*	La Palma, Canary Islands	EF446839
30535	*Sylvia atricapilla heineken*	La Gomera, Canary Islands	EF446840
30543	*Sylvia atricapilla heineken*	La Palma, Canary Islands	EF446840
30520	*Sylvia atricapilla heineken*	El Hierro, Canary Islands	EF446841
30522	*Sylvia atricapilla heineken*	El Hierro, Canary Islands	EF446841
30534	*Sylvia atricapilla heineken*	La Gomera, Canary Islands	EF446842
30533	*Sylvia atricapilla heineken*	La Gomera, Canary Islands	EF446843
30536	*Sylvia atricapilla heineken*	La Gomera, Canary Islands	EF446844
30517	*Sylvia atricapilla heineken*	Tenerife, Canary Islands	EF446845
30518	*Sylvia atricapilla heineken*	Tenerife, Canary Islands	EF446846
30513	*Sylvia atricapilla heineken*	Tenerife, Canary Islands	EF446847
30526	*Sylvia atricapilla heineken*	Gran Canaria, Canary Islands	EF446847
30527	*Sylvia atricapilla heineken*	Gran Canaria, Canary Islands	EF446848
30459	*Sylvia atricapilla heineken*	Madeira	EF446849
30460	*Sylvia atricapilla heineken*	Madeira	EF446850
30439	*Sylvia atricapilla gularis*	Azores	EF446851
30438	*Sylvia atricapilla gularis*	Azores	EF446852
30440	*Sylvia atricapilla gularis*	Azores	EF446852
30505	*Sylvia atricapilla heineken*	Portugal	EF446852
30479	*Sylvia atricapilla*	Morocco	EF446853
30504	*Sylvia atricapilla heineken*	Portugal	EF446853
30506	*Sylvia atricapilla heineken*	Portugal	EF446853
30553	*Sylvia conspicillata orbitalis*	Lanzarote, Canary Islands	EF446884
30552	*Sylvia conspicillata orbitalis*	Lanzarote, Canary Islands	EF446885
30548	*Sylvia conspicillata orbitalis*	Fuerteventura, Canary Islands	EF446886
30549	*Sylvia conspicillata orbitalis*	Fuerteventura, Canary Islands	EF446886
10815	*Sylvia conspicillata orbitalis*	Gran Canaria, Canary Islands	EF446887
10815	*Sylvia conspicillata orbitalis*	Gran Canaria, Canary Islands	EF446887
30608	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
30609	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
30610	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
30612	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446854
30613	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446854
16212	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16213	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16211	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16216	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16217	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16218	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
16226	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446854
30607	*Sylvia melanocephala leucogastra*	El Hierro, Canary Islands	EF446855
16081	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446856
30616	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
30617	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
30618	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
30619	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
16084	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
16082	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446857
30558	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30559	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30560	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30561	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30564	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30565	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30566	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30567	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858

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30568	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30569	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30570	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30571	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30573	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30574	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30576	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30577	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30578	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30579	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30580	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30581	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30582	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446858
30587	*Sylvia melanocephala melanocephala*	Morocco	EF446858
30592	*Sylvia melanocephala melanocephala*	Morocco	EF446858
30599	*Sylvia melanocephala melanocephala*	Morocco	EF446858
30602	*Sylvia melanocephala melanocephala*	Portugal	EF446858
30604	*Sylvia melanocephala melanocephala*	Portugal	EF446858
30606	*Sylvia melanocephala melanocephala*	Portugal	EF446858
30614	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
30615	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
16083	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446858
16085	*Sylvia melanocephala leucogastra*	La Gomera, Canary Islands	EF446858
10819	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
10842	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
10817	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
10818	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
10849	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446858
55	*Sylvia melanocephala melanocephala*	France	EF446858
10840	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446859
10843	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446860
10844	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446861
10846	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446862
10847	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446862
10848	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446863
30611	*Sylvia melanocephala leucogastra*	Gran Canaria, Canary Islands	EF446864
30562	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446865
30563	*Sylvia melanocephala leucogastra*	Fuerteventura, Canary Islands	EF446865
30584	*Sylvia melanocephala leucogastra*	Lanzarote, Canary Islands	EF446866
30585	*Sylvia melanocephala leucogastra*	Lanzarote, Canary Islands	EF446866
30586	*Sylvia melanocephala melanocephala*	Morocco	EF446867
30598	*Sylvia melanocephala melanocephala*	Morocco	EF446867
30593	*Sylvia melanocephala melanocephala*	Morocco	EF446868
30594	*Sylvia melanocephala melanocephala*	Morocco	EF446869
30588	*Sylvia melanocephala melanocephala*	Morocco	EF446870
30595	*Sylvia melanocephala melanocephala*	Morocco	EF446870
30596	*Sylvia melanocephala melanocephala*	Morocco	EF446870
30597	*Sylvia melanocephala melanocephala*	Morocco	EF446870
30589	*Sylvia melanocephala melanocephala*	Morocco	EF446871
30590	*Sylvia melanocephala melanocephala*	Morocco	EF446872
30600	*Sylvia melanocephala melanocephala*	Morocco	EF446873
30591	*Sylvia melanocephala melanocephala*	Morocco	EF446874
30601	*Sylvia melanocephala melanocephala*	Portugal	EF446875
30603	*Sylvia melanocephala melanocephala*	Portugal	EF446875
30605	*Sylvia melanocephala melanocephala*	Portugal	EF446875
56	*Sylvia melanocephala melanocephala*	France	EF446876
57	*Sylvia melanocephala melanocephala*	France	EF446877
58	*Sylvia melanocephala melanocephala*	France	EF446878
10261	*Sylvia melanocephala melanocephala*	France	EF446879

IPMB number	(Sub-) Species	Sampling location	Genbank Acc. Number
54	*Sylvia melanocephala melanocephala*	Greece, Crete	EF446880
10425	*Sylvia melanocephala melanocephala*	Greece, Crete	EF446881
6937	*Sylvia melanocephala melanocephala*	Greece, Crete	EF446882
10260	*Sylvia melanocephala melanocephala*	Israel	EF446883

Appendix B Variable sites of cytochrome b sequences of the European robin (*Erithacus rubecula*)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

```
                11 1111222222 2333344445 5555555555 5556667777 7777788889
 1446778903 7889123448 9268901270 1124455677 8990471335 5789902570
 5293018059 7092987062 1774354347 6953958103 8486524283 6162878233
R01_LP  CCGCATGTAA CCCCCCTCTC CAACCCGCCT TCTCCTATTA CCTTCTTTTA CTTTCTACCT
R02_LP  ?????????? ?????????? ????????.. ....T..... .......... ..........
R25_LP  .......... ......C... .......... ....T..... .......... ..........
R26_LP  .......... ......C... .......... ....T..... .......... ..........
R27_LG  .......... .......... .......... .......... .......... ..........
R28_LG  .......... .......... .......... .......... .......... ..........
R29_LG  .......... ......C... .......... ....T..... .......... ..........
R30_LG  .......... ......C... .......... ....T..... .......... ..........
R54_LG  .......... ......C... .......... ....T..... .......... ..........
R55_LG  .......... .......... .......... .......... .......... ..........
R56_LG  .......... ......C... .......... ....T..... .......... ..........
R57_LG  .......... .......... .......... .......... .......... ..........
R58_LG  .......... ......C... .......... ....T..... .......... ..........
R31_HI  .......... .......... .......... .......... .......... ..........
R32_HI  .......... ......C... .......... ....T..... .......... ..........
R33_HI  .......... .......... .......... .......... .......... ..........
R34_HI  .......... .......... .......... .......... .......... ..........
R45_HI  .......... .......... .......... .......... .......... ..........
R46_HI  .......... .......... .......... .......... .......... ..........
R47_HI  .......... .......... .......... .......... .......... .C........
R48_HI  .....?.... ......C... .......... ....T..... .......... ..........
R49_HI  .......... .......... .......... .......... .......... ..........
R50_HI  .......... .......... .......... .......... .......... .C........
R51_HI  .......... .......... .......... .......... .......... ..........
R52_HI  ??????.... ......C... .......... ....T..... .......... ..........
R53_HI  .......... ......C... .......... ....T..... .......... ..........
R05_TF  TAAT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R06_TF  TA...CACG. TTT.T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R07_TF  T....CACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CCG .CA...G..C
R08_TF  T....CACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R11_TF  T.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R12_TF  ????????G. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R14_TF  TA....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R15_TF  T....CACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R16_TF  T.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R17_TF  ?.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R18_TF  T.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R19_TF  T.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R20_TF  T.....ACG. TT..T.AT.T .GGTATAT.C C.CT..GA.. T...TC.CC. .CA...G..C
R21_TF  T.....ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R22_TF  ?????????? TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R23_TF  T...T.ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CC. .CA...G..C
R24_TF  ???...ACG. TT..T.AT.T .G.TATAT.C ..CT..GA.. T...TC.CCG .CA...G..C
R03_GC  ????????.G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R04_GC  T.AT..A..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R35_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG .TCCT.CCC. ..ACTC.TTC
R36_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R37_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R38_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG .T.CT.CCC. ..ACTC.TTC
R59_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG .T.CT.CCC. ..ACTC.TTC
R60_GC  T.AT..A..G .T.T.TA.C. TG....A.T. C.C..C.ACG .T.CT.CCC. ..ACTC.TTC
R61_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R62_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R63_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R64_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R65_GC  T.AT.CA..G .T.T.TA.C. TG....A.T. C.C..C.ACG TT.CT.CCC. ..ACTC.TTC
R10_D   .......... .......... .......... .T........ .......... T.........
R39_FU  .......... .......... .......... .T........ .......... T.........
R40_FU  .......... .......... .......... .T...C.... .......... T.........
R41_P   .......... .......... .......... .......C.. .......... ..........
R42_P   .......... .......... .......... .......C.. .......... ..........
R43_P   .......... .......... .......... .......C.. .......... ..........
R44_P   .......... .......... .......... .......... .......... ..........
```

```
                    111111111 11111111
          9999999999 9000000000 00000000
          3556677788 9000001222 33344566
          2145603829 3023571156 12547303

R01_LP    TCTTCATCAG CTTATTCAAA TCACTTTG
R02_LP    .........T .......G.. ........
R25_LP    C.CC.....T .......... ......??
R26_LP    C.CC.....T .......... .A.....?
R27_LG    C.CC.....T .......... .A..C.CA
R28_LG    C.CC.....T .......... .A..C.CA
R29_LG    C.CC.....T .......... .A..C.CA
R30_LG    C.CC.....T .......... .A..C.CA
R54_LG    C.CC.....T .......... .A..C.CA
R55_LG    C.CC.....T .......... .A..C.CA
R56_LG    C.CC.....T .......... .A..C.CA
R57_LG    C.CC.....T .......... .A..C.CA
R58_LG    C.CC.....T .......... .A..C.CA
R31_HI    C.CC.....T .......... .A..C..A
R32_HI    C.CC.....T .......... .A..C.CA
R33_HI    C.CC.....T .......... .A..C.CA
R34_HI    C.CC.....T .......... .A..C.CA
R45_HI    C.CC.....T .......... .A..C.CA
R46_HI    C.CC.....T .......... .A..C.C?
R47_HI    C.CC.....T .......... .A..C.CA
R48_HI    C.CC.....T .......... .A..C.CA
R49_HI    C.CC.....T .......... .A..C.CA
R50_HI    C.CC.....T .......... .A..C.CA
R51_HI    C.CC.....T .......... .A..C.CA
R52_HI    C.CC.....T .......... .A..C.CA
R53_HI    C.CC.....T .......... .A..C.CA
R05_TF    CTCC.GC.GT T.C....... .A..C.C.
R06_TF    C.CCTGC..T ..C.C....G .T.TC...
R07_TF    C.CCTGC..T ..C.C....G .T.TC...
R08_TF    C.CCTGC..T ..C.C....G CTCTC...
R11_TF    C.CC.....T ...CA..... ....C...
R12_TF    C.CC.....T ...CA..... ....C...
R14_TF    C.CC.....T ...CA..... ....C...
R15_TF    C.CC.G...T ...CA..... ....C...
R16_TF    C.CC.G...T ...CA..... ....C...
R17_TF    C.CC.G...T ...CA..... ....C...
R18_TF    C.CCTGC..T ...CA..... ....C...
R19_TF    C.CCTGC..T ...CA..... ....C...
R20_TF    C.CCTGC.GT ...CA..... ....C...
R21_TF    C.CCTGC..T ...CA..... ....C...
R22_TF    C.CCTGCT.T ...CA..... ....C...
R23_TF    C.CCTGC.GT ...CA..... ....C...
R24_TF    C.CCTGC..T ...CA..... ....C...
R03_GC    CTCC.GC.GT T.C......G .T..CC..
R04_GC    CTCC.GC.GT T.C......G .T..CC..
R35_GC    CTCC.GC.GT T.C.....C. .A..C.??
R36_GC    CTCC.GC.GT T.C....... .A..C.CA
R37_GC    CTCC.GC.GT T.C...A... .A..C.CA
R38_GC    CTCC.GC.GT T.C....... .A..C.CA
R59_GC    C.CC.GC.GT T.C....... .A..C.CA
R60_GC    CTCC.GC.GT T.C....... .A..C.CA
R61_GC    CTCC.GC.GT T.C...A... .A..C.C?
R62_GC    CTCC.GC.GT T.C...A... .A..C.CA
R63_GC    CTCC.GC.GT T.C....... .A..C.C.
R64_GC    CTCC.GC.GT ..C..C.... .A..C.C?
R65_GC    CTCC.GC.GT T.C....... .A..C.CA
R10_D     C.CCT.C..T ....C..... ....C...
R39_FU    C.CC.....T ....C..... .A..C.CA
R40_FU    C.CC.....T ....C..... .A..C.CA
R41_P     C.CC.....T .......... .A..C.CA
R42_P     C.CC.....T .C........ .A..C.CA
R43_P     C.CC.....T .C........ .A..C.CA
R44_P     C.CC.....T .......... .A..C.CA
```

Appendix C Variable sites of cytochrome b sequences of goldcrests (*Regulus* sp.)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

```
                                                                    1111111 1111111111
                          111222333 4445555555 6666777778 8990011111 2222233333
                         9256123249 6891456789 0346012451 4032824678 0178956789

R. teneriffae (La_Palma) GAATCAGTTA ACGCCACCTA CCAATTATTC CACCCACTCC CATCAAATTG
R. teneriffae (El Hierro) A......... .......... .......... .......... ..........
R. teneriffae (La Gomera) A......... .......... ......G... ......T... ..........
R. teneriffae (Tenerife) A......... .......... ......G... ......T... ..........
R. azoricus (Azores)     ...C...... G......... ...G...... .......... ..........
R. inermis (Azores)      ...C...... G......... ...G...... .......... ..........
R. regulus (Europe)      ...C...... G......... ...G...... .......... ..........
R. madeirensis (Madeira) AG.C..A... .A..T..T.T ..T.A...C. ......T.T. .C....G.AA
R. ignicapillus (Europe) AG.CTGAC.. .AA.T..T.T ....GC..CT TGA..GT.T. TT......AA
R. calendula (N-Amerika) A.TC..A.GG .T.T.GAAC. TA..A..CC. ...A...... A.A.TG.C.A

                         1111111111 1111122222 2222222222 2333333333 3333333333
                         4466677778 8999900112 3344555678 9001122222 3333344556
                         1224814584 7258947392 1406356128 3032514567 0367925143

R. teneriffae (La_Palma) CTTACCTCAA ACTTCCTTAG AAACCCTATA TGACACCAAC CCCTCCTCGT
R. teneriffae (El Hierro) .......... .......... .......... .......... ..........
R. teneriffae (La Gomera) .......... ........C. ..G....... .......... .........C
R. teneriffae (Tenerife) .......... .......... ..G....... .......... .........C
R. azoricus (Azores)     .......... G......... .......... .......... ..T.......
R. inermis (Azores)      .......... G......... .......... .......... ..T.......
R. regulus (Europe)      .......... G......... .......... .......... A.........
R. madeirensis (Madeira) .....TAT.. .TC.TT.C.A ...T.....C .ATTGTT..T AA..T.CTA.
R. ignicapillus (Europe) .C.GT.AT.. ..C.T..C.A T........T .ACT..T... AA...TCTAC
R. calendula (N-Amerika) T.C.T.A.CG .TCC..C..A TTG.TACC.C ..TT...TC. A.TG..C.AC

                         3333333333 4444444444 4444444555 5555555555 5555555556
                         7788889999 0001233445 5567788000 0122223344 4456788990
                         2914670369 0284958141 3951403147 8435681703 6981358473

R. teneriffae (La_Palma) CCCCATCCCA ACATCCTTTC TCCCTATCCC CCACGTCCCC AAGACCTATC
R. teneriffae (El Hierro) ....G..... .........T .......... .......... ..........
R. teneriffae (La Gomera) ....G..... .........T ...A.G.... .......... ...G......
R. teneriffae (Tenerife) ....G..... .........T ...A.G.... .......... ...G......
R. azoricus (Azores)     ....G..TT. ........CT ...A...... .......... ..........
R. inermis (Azores)      ....G..TT. ........CT ...A...... .......... ..........
R. regulus (Europe)      ....G..TT. ........CT ...A...... .......... ..........
R. madeirensis (Madeira) T.T.GAT.T. .TCC...CCT CTTACGC... .T.T.CTT.. GTAG.TCTCT
R. ignicapillus (Europe) T.T.GA.AT. GTCC..CC.T C...C.CTA. .T.A.C.... .CAGA..TCT
R. calendula (N-Amerika) .TATGC...C ..TCTTCCCT CTTAC.C..A T....C.GTT .CAG..CTCT

                         6666666666 6666666666 6667777777 7777777777 7777777777
                         0011223344 5556677889 9990000111 2222333444 5566667777
                         4928173658 1473925470 2362589017 0369268147 0912581247

R. teneriffae (La_Palma) AATAAGAATA CTCCTCTCTG ACCAACGTCA CGTGCCACCA CGCTCTCCGT
R. teneriffae (El Hierro) .......... .......... .......... .......... ..........
R. teneriffae (La Gomera) ..C....... .C.......T ......C... .C........ .A......A.
R. teneriffae (Tenerife) ..C....... .C.......T ......C... .C........ .A......A.
R. azoricus (Azores)     .......... ........CA .......... .C.A...... .A......A.
R. inermis (Azores)      .......... ........CA .......... .C.A...... .A......A.
R. regulus (Europe)      ..C....... .........A .......... .C.A...... .A......A.
R. madeirensis (Madeira) .TCC.AG.GC .CA.ATC.CA ........A. .A.A.T.T.. TA.CT.ATAA
R. ignicapillus (Europe) .CCTGA.GAT .CA.ATCTCA ...T..C.AT .A.A.T.... .A.C.CA..A
R. calendula (N-Amerika) ..CC....A. TCATAAC.CA .T.C.T.... ACCAA.G..C .A.C.CA.CA
```

```
                                                                                                  11111
                                 7777788888 8888888888 8888888999 9999999999 9999900000
                                 8889901233 4455555668 8889999012 3344556677 8899900011
                                 0392513224 0602568272 4581457084 0625170658 4706925914

R. teneriffae (La_Palma)         CACTCCCCTC AGAGTGCCCT CAACCTAATG AAAATTACTA CCAACGCCTG
R. teneriffae (El Hierro)        .......... .......... .......... .......... ..........
R. teneriffae (La Gomera)        .......... ......T... .....CG... ....CC...G .....AT.GA
R. teneriffae (Tenerife)         .......... ......T... .....CG... ....CC...G .....AT.GA
R. azoricus (Azores)             T......... .....AT... .....C...T ....C..... .....A..GA
R. inermis (Azores)              T......... .....AT... .....C...T ....C..... .....A..G.
R. regulus (Europe)              T......... .....AT... .....C...T ....C..... .....A..G.
R. madeirensis (Madeira)         ....T.TT.. GATA.AT.TC ..T.T..CCT .GCG.G.TC. ...TTAT.GA
R. ignicapillus (Europe)         ...CTT.T.T GATAC..TTC ..T.T..CCC TG...A.... TT.T.A..A.
R. calendula (N-Amerika)         .CTC...TCA .A.ACAT..C .CT.AC.CAC C.T..C..C. ..TC.ATTAA

                                 1111111111 1111111111 1111111111 1111111111 111111
                                 0000000000 0000000000 1111111111 1111111111 222222
                                 1222333445 5566777789 0001112334 5566788899 011112
                                 7016258170 6958146732 1470696476 2514024847 635781

R. teneriffae (La Palma)         ACCTTAGACG CGGACCCTGA TGGACGAACA ACCCATCCCT ATCACC
R. teneriffae (El Hierro)        .......... .......... .......... .......... ...C..
R. teneriffae (La Gomera)        ......A..A ..A......G ..A...G... .....C.... .CTC..
R. teneriffae (Tenerife)         ......A..A ..A......G ..A...G... .....C.... ..TC..
R. azoricus (Azores)             ......A..A .........G ..A....... .......... ...C..
R. inermis (Azores)              ......A..A .A.......G ..A....... .......... ...C..
R. regulus (Europe)              ......A..A .........G .......... .......... ...C..
R. madeirensis (Madeira)         GATC..T..A TAT.T..CAG CACCTA.TT. ..T..C..T. ...C.T
R. ignicapillus (Europe)         .ATC..C.TA ..C....CAG CACC.A.C.C ..T..C.TT. ...C..
R. calendula (N-Amerika)         ...CCTAC.A .A.T.A.AA. .CC..A.C.C CT.AGC..TC GA.C..
```

Appendix D Variable sites of cytochrome b sequences of the blue tit (*Parus teneriffae* - group)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

Sample no. Alignment position

```
                                                                                                                      1
                    111222222222233333333333334444444444445555556666666666677777777777778888888888888899999999999999990
          1236667789489234444679900123345667890002235566792457890023335678900022445688990344556666778800112345777889990
         76603693519765670679494739273991362102680666928788986853770134351625603479836581736031245233806287094235173452
La Gomera
16087LG  GAGGCATGTTCTACGCTGGTATGGCTTTCAATGCGCGCCACGCGAAACCTACCGCACCCCACTAAGCCCCCAAATCAGCCCGCATAATCCTGAGACCTTCTAATCTCATA
16088LG  ..............................................................................................................
16097LG  ..............A...............................................................................................
30175LG  .......A....G.............C.........................................T.........................................
30176LG  ..............................................................................................................
30177LG  ..............................................................................................................
30178LG  ..............................................................................................................
30179LG  .........................................................A....................................................
30181LG  ..............A...............................................................................................
30182LG  .........................................................A....................................................

Tenerife
30185TF  .......A..........................................................A..T.........................................
30188TF  .C.....A..........................................................A..T.........T...............................
30190TF  .C.....A..........................................................A..T.........................................
30184TF  .C.....A..........................................................A..T.........................................
30187TF  .C.....A..........................................................A..T.........................................
30189TF  .C.....A..........................................................A..T.........................................
30183TF  .C.....A.............................................................T.........T...............................
30186TF  .C.....A.............................................................T.........T...............................

Gran Canaria
30164GC  .......A....G.............C..............................T..........T..G.........A...............C....G.......
30165GC  .......A....G.............C..............................T..........T..G.........A.............A.C....G.......
30166GC  ...A...A....G.............C..............................T..........T..G...TG....A...............C....G.......
30167GC  ...A...A....G.............C..............................T..........T..G...TG....A.......T.....A.C....G.......
30169GC  ...A...A....G.............C..............................T..........T..G...TG....A...............C....G.......
30170GC  .......A....G.............C..............................T..........T..G.........A.............A.C....G.......
30171GC  ...A...A....G.............C..............................T..........T..G...TG....A.............A.C....G.......
30172GC  .......A....G.............C..............................T..........T..G...T.....A.............A.C....G.......
30173GC  ...A...A....G.............C..............................T..........T..G...TG....A.............A.C....G.......

El Hierro
30158HI  .......A.C...................G.C..A..T.G..T.....T...TA.G...T.....A......G..T.A...A............G..C....G..C....
30159HI  .......A.C...................G.C..A..T.G..T.....T...TA.G...T.....A......G..T.A...A............G..C....G..C....
30161HI  .......A.C...................G.C..A..T.G..T.....T...TA.G...T.....A......G..T.A...A............G..C....G..C....
32842HI  .......A.C...................G.C..A..T.G..T.....T...TA.G...T.....A......G..T.A...A............G..C....G..C....
16207HI  .......A.C...................G.C..A..T.G..T.....T...TA.G...T.....A......G..T.A...A............G..C....G..C....
30160HI  .......A.C...................G.C..A..T.G..T.....T...TA.G.T.T.....A......G..T.A...A............G..C....G..C....

La Palma
32836LP  ...A..CAC.T....T.A..GC...CCC..GCA.A.A....A.AC.....CT.A...T....C..A...T......GA..T...C.GC..C..A...C..CG....TGC.
32837LP  ...A..CAC.T....T.A..GC...CCC..GCA.A.A....A.AC.....CT.A...T....C..A...T......GA..T...C.GC..C..A...C..CG....TGC.
10357LP  ...A..CAC.T....T.A..GC...CCC..GCA.A.A....A.AC.....CT.A...T....C..A...T......GA..T...C.GC..C..A...C..CG....TGC.
30960LP  ...A..CAC.T....T.A..GC...CCC..GCA.A.A....A.AC.....CT.A...T....C..A...T......GA..T...C.GC..C..A...C..CG....TGC.
33500LP  ...A..CAC.T....T.A..GC...CCC..GCA.A.A....A.AC.....CT.A...T....C..A...T......GA..T...C.GC..C..A...C....G.......

Fuerteventura
30143FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30130FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30136FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C.T..G...T...
30137FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C.T..G...T...
30138FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TTT.....T..T..A...G.C.........C.T..G...T...
30139FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TTT.....T..T..A...G.C.........C.T..G...T...
30140FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C.T..G...T...
30141FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TTT.....T..T..A...G.C.........C.T..G...T...
30142FU  T.....CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TTT.....T..T..A...G.C.........C.T..G...T...

Lanzarote
30144LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30148LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30151LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30145LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30146LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30154LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...
30156LA  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C...TTT......T..T..A...G.C.........C.T..G...T...

Morocco
30119MK  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C....G...T...
30120MK  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C....G...T...
30121MK  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C....G...T...
30122MK  ......CA.........A....A...C.T...A.A..T...A..............TT.TG.C....TT......T..T..A...G.C.........C....G...T...

Portugal
30123P   ..TATGCAC..C.TA.C..C.C.AT..C...CATAGA.T.T...C.CT.CC..AAC..TT.TCCTA....T..G...A.AAATGC..CT..AG...TCC.C.G.T....G
30124P   ..TATGCAC..C.TA.C..C.C.AT..C...CATAGA.T.T...C.CT.CC..AAC..TT.TCCTA....T..G...A.AAATGC..CT..AG...TCC.C..CT....G

France
30969F   ..TA.GCAC..C.TA.C.AC.CAAT..C...CATAGA.T.T..AC.CT.CC..AAC..TT.TCCTA.......GC..A.AAATGC..CT..AG...TCC.C.GCT....G
30968F   ..TA.GCAC..C.TA.C.AC.CAAT..C...CATAGA.T.T..ACGCT.CC..AAC..TT.TCCTA.......GC..A.AAATGC..CT..AG...TCC.C.GCT....G
30970F   ..TA.GCAC..C.TA.C.AC.CAAT..C...CATAAA.T.T..AC.CT.CC..AAC..TT.TCCTA.......GC..A.AAATGC..CT..AG...TCC.C.GCT....G
```

Appendix E Variable sites of cytochrome b sequences of the Sardinian warbler (*Sylvia melanocephala*)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

Haplot.	N	Alignment position					
		11	1111122233	3333344445	5566666777	7888888899	99
		1333578901	4788912304	4577803663	7903449244	4011566900	11
		1018235402	3214943843	6539533392	7242014125	8557329517	03
Hm01	38	GACCCCTTCC	CGATTCCTGG	ACTATCCGAC	TTTGCCCATT	TTCAACGACT	CA
Hm02	1	....	.....T...A	....	....	.....T....	..
Hm03	1	...T..C...	A.G..T....	..CG......	C	C...GT....	..
Hm04	1	....	....	....	....	.CT.......	..
Hm05	2	...T.TCC..	.AG..T.CAA	..CGCT..GT	..C...TGCC	CCT.GT....	..
Hm06	1	...T.TCC..	AAG..T.CAA	..CGCT..GT	..C...TGCC	C.T.GT....	..
Hm07	1	....	.....T....	....	....	.....T....	..
Hm08	12	........TT	.....T...A	.T........	....	....	..
Hm09	2	....	....	....	....	....	.C
Hm10	1	...T......	.....T....	....	....	C.........	..
Hm11	6	....	...CC.....	.......A..	....	....	..
Hm12	2	....	....	....	......T...	....	..
Hm13	3	....	...C......	.......A..	....	....	..
Hm14	1	........TT	.....T...A	GT........	....	....	..
Hm15	1	....	....	....	.C........	....	..
Hm16	1	....	....	....	.C........	.......CTC	A.
Hm17	1	C.........	....	.......A..	...AGG....	.......CTC	A.
Hm18	1	.......C.T	.AG..T..A.	.......A..	C.........	C...G..CTC	A.
Hm19	1	....	.........A	.......A..	....	....	..
Hm20	1	....	....	.......A..	....	....	..
Hm21	1	....T.....	....	.......A..	....	....	..
Hm22	1	.CA.......	....	....	....	....	.C
Hm23	2	........TT	.....T...A	....	....	....	..
Hm24	1	....	....	......T...	....	...G......	..
Hm25	1	......C...	....	....	....	....	..
Hm26	4	....	....	......T...	....	....	..
Hm27	1	........TT	.....T....	....	....	....	..
Hm28	1	....T.....	......T...	......T...	....	....	..
Hm29	1	....	.....T....	......T...	....	....	..
Hm30	1	........TT	.....T...A	....	....	..T...A...	.G

Appendix F Variable sites of cytochrome b sequences of the blackcap (*Sylvia atricapilla*)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

Haplot.	N	Alignment position
		22333455 667
		1413469645 271
		6015844953 202
Ha01	2	AGCTCGCGTC CAG
Ha02	2	........T. ...
Ha03	1	.... TGA
Ha04	1	C.......T. ...
Ha05	1	......T.T. ...
Ha06	4	C.......TC ...
Ha07	1	CA........ ...
Ha08	1	C......... TGA
Ha09	2	C......C.. ..A
Ha10	1	..TC...... ...
Ha11	1	....T..... ..A
Ha12	1	....T..... ...
Ha13	3	C......... ..A
Ha14	3	C......... ...
Ha15	1	C....A.... ..A

Appendix G Variable sites of cytochrome b sequences of the spectacled warbler (*Sylvia conspicillata*)

Numbers above each column indicate the position in the corresponding gene according to the chicken mitochondrial genome Desjardins & Morais 1990).

Haplot.	N	Alignment position
		466778
		1845036
		6705035
Hc01	2	CGCCGCC
Hc02	2	AATAAT.
Hc03	1	AA....T
Hc04	1	AA.AA..

Appendix H **Comparison of morphometric measurements of Sylvia melanocephala from different populations**

Measurements are compared for birds from the western Canary Islands (El Hierro, La Gomera, Gran Canaria), eastern Canary Islands (Fuerteventura, Lanzarote), Europe (Portugal) and North Africa (Morocco). Significant mean differences from pairwise ANOVA comparisons are listed.

♂	[1] Western Canary Islands				[2] Eastern Canary Islands				[3] Morocco				[4] Portugal			
	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from
Wing	6	57.6 ± 1.2	56.0 – 59.0	[2]** [3]** [4]**	33	59.6 ± 1.4	56.6 – 62.0	[1]**	7	60.0 ± 1.0	58.0 – 61.0	[1]**	4	60.0 ± 2.0	58.5 – 63.0	[1]**
P9	6	39.3 ± 1.0	37.5 – 40.5	[2]* [4]**	29	41.1 ± 1.9	37.5 – 46.0	[1]* [4]*	7	41.0 ± 1.0	39.5 – 42.5	–	3	43.5 ± 4.1	40.0 – 48.0	[1]** [2]*
P8	6	43.9 ± 1.3	42.0 – 46.0	[2]** [3]** [4]*	28	45.7 ± 1.3	43.0 – 49.0	[1]**	7	46.1 ± 0.2	46.0 – 46.5	[1]**	3	45.8 ± 2.0	44.0 – 48.0	[1]*
P7	6	45.6 ± 1.2	44.0 – 47.0	[2]* [3]*	28	47.1 ± 1.5	44.0 – 51.0	[1]*	7	47.5 ± 0.3	47.0 – 48.0	[1]*	3	47.2 ± 1.3	46.0 – 48.5	–
P6	6	46.7 ± 1.5	45.0 – 49.0	–	28	47.5 ± 1.4	44.5 – 50.5	–	7	48.1 ± 0.3	47.5 – 48.5	–	3	47.7 ± 1.8	46.0 – 49.5	–
P5	6	46.5 ± 1.5	45.0 – 48.5	–	30	46.7 ± 1.6	42.5 – 49.0	–	7	47.4 ± 0.3	47.0 – 48.0	–	3	47.0 ± 0.5	46.5 – 47.5	–
P4	6	45.4 ± 1.6	43.0 – 47.0	–	30	45.9 ± 1.4	42.5 – 48.0	–	7	46.4 ± 0.4	46.0 – 47.0	–	3	46.0 ± 1.0	45.0 – 47.0	–
P3	6	44.8 ± 1.6	42.0 – 46.0	–	30	45.2 ± 1.2	42.0 – 47.5	–	7	45.8 ± 0.6	44.5 – 46.5	–	3	45.3 ± 0.3	45.0 – 45.5	–
P2	6	44.5 ± 1.5	42.0 – 46.0	–	30	44.9 ± 1.3	42.0 – 48.0	–	7	45.7 ± 0.4	45.0 – 46.0	–	3	44.3 ± 0.8	43.5 – 45.0	–
P1	6	43.8 ± 1.4	41.5 – 45.5	–	29	44.3 ± 1.2	42.0 – 46.0	–	7	45.0 ± 0.5	44.0 – 45.5	–	3	44.5 ± 1.3	43.0 – 45.5	–
S1	6	44.1 ± 1.0	43.0 – 45.5	–	30	43.3 ± 1.4	40.5 – 46.0	–	7	44.1 ± 0.5	43.5 – 45.0	–	3	42.8 ± 1.8	41.0 – 44.5	–
Tarsus	6	19.9 ± 0.6	19.3 – 20.9	[2]*	31	19.4 ± 0.5	18.4 – 20.5	[1]*	7	19.6 ± 0.5	18.7 – 20.3	–	3	19.2 ± 0.3	19.0 – 19.6	–
NaLoSpi	5	7.5 ± 0.4	7.1 – 8.0	[2]** [3]*	31	7.0 ± 0.3	6.1 – 7.6	[1]**	7	7.1 ± 0.4	6.4 – 7.4	[1]*	3	7.1 ± 0.3	6.9 – 7.4	–
Bill length	6	15.8 ± 0.8	15.0 – 17.2	[4]*	31	15.3 ± 0.6	14.0 – 16.6	–	7	15.4 ± 0.9	14.1 – 16.7	–	3	14.9 ± 0.5	14.4 – 15.3	[1]*
Bill heigt	5	3.1 ± 0.2	2.9 – 3.3	–	31	3.0 ± 0.1	2.8 – 3.3	–	7	3.0 ± 0.3	2.8 – 3.7	–	3	3.2 ± 0.2	3.0 – 3.4	–
Bill width	5	4.5 ± 0.3	4.1 – 4.8	[4]*	31	4.5 ± 0.3	3.8 – 5.0	[4]**	7	4.3 ± 0.5	3.6 – 5.0	[4]***	3	5.1 ± 0.5	4.8 – 5.7	[1]* [2]** [3]***
Foot in	5	22.4 ± 0.4	22.0 – 23.0	[2]** [3]* [4]**	31	21.4 ± 0.5	20.5 – 22.0	[1]**	6	21.7 ± 1.0	21.0 – 23.5	[1]*	3	21.2 ± 0.3	21.0 – 21.5	[1]**
Foot mid	5	28.1 ± 0.5	27.5 – 29.0	[2]** [3]* [4]*	31	27.2 ± 0.6	26.0 – 28.5	[1]**	6	27.1 ± 1.0	26.0 – 29.0	[1]*	3	26.8 ± 0.3	26.5 – 27.0	[1]*
Foot out	5	23.3 ± 0.4	23.0 – 24.0	[2]** [3]* [4]**	31	22.1 ± 0.6	21.0 – 23.0	[1]**	6	22.4 ± 1.0	21.0 – 24.0	[1]*	3	21.7 ± 0.6	21.0 – 22.0	[1]**

♀	[1] Western Canary Islands				[2] Eastern Canary Islands				[3] Morocco				[4] Portugal			
	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from	n	mean ± s.d.	range	Signif. from
Wing	5	56.5 ± 1.4	55.0 – 58.0	[2]** [3]** [4]***	28	58.9 ± 1.5	56.0 – 63.0	[1]**	8	58.8 ± 1.8	57.0 – 62.0	[1]**	2	61.0 ± 0.0	61.0 – 61.0	[1]***
P9	5	37.7 ± 1.1	36.0 – 39.0	[2]** [3]** [4]*	23	39.5 ± 1.1	38.0 – 42.5	[1]**	8	40.1 ± 1.5	37.5 – 42.5	[1]**	2	40.3 ± 1.1	39.5 – 41.0	[1]*
P8	5	42.6 ± 1.1	41.0 – 43.5	[2]*** [3]** [4]**	25	44.9 ± 1.3	43.0 – 48.5	[1]***	8	45.1 ± 1.5	43.0 – 47.5	[1]**	2	46.0 ± 0.0	46.0 – 46.0	[1]**
P7	5	44.0 ± 1.2	42.0 – 45.0	[2]*** [3]** [4]**	25	46.3 ± 1.2	44.0 – 48.5	[1]***	8	46.2 ± 1.8	43.5 – 49.0	[1]**	2	47.5 ± 0.0	47.5 – 47.5	[1]**
P6	5	45.0 ± 1.1	43.5 – 46.0	[2]*** [3]** [4]**	26	47.0 ± 1.0	45.0 – 49.0	[1]***	8	46.9 ± 1.6	45.0 – 49.0	[1]**	2	48.3 ± 0.4	48.0 – 48.5	[1]**
P5	5	45.2 ± 1.3	44.0 – 47.0	[2]* [3]* [4]**	25	46.4 ± 0.9	44.0 – 47.5	[1]*	8	46.6 ± 1.4	44.5 – 48.5	[1]*	2	47.8 ± 0.4	47.5 – 48.0	[1]**
P4	5	44.9 ± 1.4	43.0 – 46.5	–	26	45.4 ± 1.2	41.5 – 47.0	–	8	45.9 ± 1.0	44.5 – 47.0	–	2	46.5 ± 0.0	46.5 – 46.5	–
P3	5	44.5 ± 1.7	43.0 –46.5	–	25	44.7 ± 1.1	42.0 – 47.0	–	8	45.2 ± 1.0	43.5 – 46.0	–	2	45.5 ± 0.0	45.5 – 45.5	–
P2	5	44.2 ± 1.7	42.5 – 46.0	–	25	44.4 ± 1.0	42.0 – 46.5	–	8	44.9 ± 1.1	43.0 – 46.0	–	2	45.5 ± 0.0	45.5 – 45.5	–
P1	5	43.7 ± 1.9	42.0 – 46.0	–	25	43.8 ± 1.0	42.0 – 46.5	–	8	44.1 ± 1.4	42.0 – 46.0	–	2	45.0 ± 0.0	45.0 – 45.0	–
S1	5	43.5 ± 1.8	41.5 – 45.5	–	26	42.8 ± 1.0	41.0 – 45.0	–	8	43.5 ± 1.0	42.0 – 45.0	–	2	43.3 ± 0.4	43.0 – 43.5	–
Tarsus	5	19.9 ± 0.5	19.3 – 20.5	[2]*	26	19.3 ± 0.4	18.5 – 20.2	[1]* [3]*	8	19.7 ± 0.5	19.1 – 20.3	[2]*	2	19.7 ± 1.1	18.9 – 20.5	–
NaLoSpi	4	7.3 ± 0.3	6.8 – 7.6	–	25	7.2 ± 0.4	6.1 – 7.8	–	8	7.0 ± 0.2	6.6 – 7.3	–	2	7.2 ± 0.4	6.9 – 7.4	–
Bill length	5	15.0 ± 0.8	14.2 – 16.2	–	25	15.3 ± 0.5	14.5 – 16.4	–	8	15.3 ± 0.7	14.1 – 16.2	–	2	15.8 ± 0.8	15.2 – 16.3	–
Bill heigt	4	3.1 ± 0.1	3.0 – 3.2	–	26	3.1 ± 0.1	2.7 – 3.3	[3]*	8	2.9 ± 0.0	2.8 – 3.1	[2]*	2	3.1 ± 0.0	3.1 – 3.1	–
Bill width	4	4.3 ± 0.7	3.6 – 4.9	[4]*	26	4.4 ± 0.4	3.5 – 5.2	[4]*	8	4.2 ± 0.4	3.3 – 4.4	[4]**	2	5.2 ± 0.5	4.8 –5.5	[1]* [2]* [3]**
Foot in	4	22.4 ± 0.5	22.0 – 23.0	[2]* [3]** [4]*	26	21.4 ± 0.6	20.0 – 22.0	[1]*	8	21.1 ± 1.0	20.0 – 23.0	[1]**	2	21.0 ± 1.4	20.0 – 22.0	[1]*
Foot mid	4	27.9 ± 1.0	26.5 – 29.0	[3]***	26	27.1 ± 0.5	26.0 – 28.0	[3]**	8	26.1 ± 1.2	24.0 – 27.5	[1]*** [2]**	2	27.0 ± 1.4	26.0 – 28.0	–
Foot out	4	23.0 ± 0.0	23.0 – 23.0	–	26	22.5 ± 2.0	20.5 – 32.0	–	7	21.7 ± 1.0	20.0 – 23.0	–	2	21.8 ± 1.1	21.0 – 22.5	–

Appendix I Comparison of morphometric measurements of *Sylvia atricapilla* from different populations

Measurements are compared for birds from the Azores, Madeira, Canary Islands, North Africa (Morocco) and Europe (Portugal). Significant mean differences from pairwise ANOVA comparisons are listed.

♂	[1] Azores (n = 7)			[2] Madeira (n = 10)			[3] Canary Islands (n = 13)			[4] Morocco (n = 14)			[5] Portugal (n = 5)		
	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from
Weight	17.7 ± 1.6	15.0 – 19.2	[2]*** [3]*	14.9 ± 0.6	13.8 – 15.7	[1]*** [3]*** [4]*** [5]***	16.6 ± 0.9	15.6 – 18.8	[1]* [2]*** [4]**	17.7 ± 1.0	16.2 – 18.9	[2]*** [3]**	16.9 ± 1.0	15.9 – 18.2	[2]***
Wing	72.4 ± 2.5	67.0 – 74.0	–	71.5 ± 1.9	69.0 – 76.0	[4]** [5]*	71.7 ± 1.6	69.0 – 74.5	[4]** [5]*	74.0 ± 1.8	71.5 – 76.0	[2]** [3]**	73.9 ± 1.0	73.0 – 75.0	[2]* [3]*
P9	49.5 ± 1.7	48.0 – 52.0	[3]*	48.4 ± 1.6	45.5 – 51.5	[4]*** [5]**	47.9 ± 1.4	46.0 – 51.0	[1]* [4]*** [5]***	50.6 ± 1.7	48.0 – 53.0	[2]*** [3]***	51.0 ± 0.0	51.0 – 51.0	[2]** [3]***
P8	56.9 ± 0.7	56.0 – 58.0	[3]***	55.9 ± 1.6	54.0 – 60.0	[3]** [5]**	54.3 ± 1.1	52.0 – 56.0	[1]*** [2]** [4]*** [5]***	56.9 ± 1.3	54.5 – 59.0	[3]***	58.0 ± 0.0	58.0 – 58.0	[2]** [3]***
P7	58.8 ± 1.2	57.5 – 61.0	[2]** [3]***	56.9 ± 2.0	55.0 – 62.0	[1]** [3]**	55.3 ± 1.1	53.5 – 57.0	[1]*** [2]** [4]*** [5]**	57.9 ± 1.4	55.5 – 60.0	[3]***	57.5 ± 0.0	57.5 – 57.5	[3]**
P6	58.2 ± 1.2	56.5 – 59.5	[2]* [3]*** [4]*	56.5 ± 1.9	55.0 – 61.5	[1]* [3]*	54.9 ± 1.5	53.0 – 58.0	[1]*** [2]* [4]** [5]**	56.6 ± 1.4	54.0 – 58.0	[1]* [3]**	57.0 ± 0.0	57.0 – 57.0	[3]**
P5	55.4 ± 1.0	54.0 – 57.0	[2]** [3]*** [4]**	53.4 ± 1.8	52.0 – 58.0	[1]**	52.2 ± 1.7	50.0 – 56.0	[1]*** [4]* [5]**	53.4 ± 1.3	51.5 – 55.5	[1]** [3]*	54.5 ± 0.0	54.5 – 54.5	[3]**
P4	54.0 ± 0.8	52.5 – 54.5	[2]*** [3]*** [4]*** [5]***	51.6 ± 1.4	50.0 – 55.0	[1]***	50.5 ± 1.4	48.5 – 53.0	[1]*** [4]*	51.6 ± 1.2	49.5 – 53.0	[1]*** [3]*	51.0 ± 0.0	51.0 – 51.0	[1]***
P3	53.1 ± 1.5	50.5 – 54.0	[2]** [3]*** [4]**	51.1 ± 1.6	49.0 – 55.0	[1]** [3]*	49.7 ± 1.5	47.5 – 52.5	[1]*** [2]* [4]* [5]***	51.0 ± 1.2	49.5 – 53.0	[1]** [3]** [5]*	52.5 ± 0.0	52.5 – 52.5	[3]*** [4]*
P2	52.4 ± 0.9	51.0 – 53.5	[2]*** [3]*** [4]*** [5]*	50.2 ± 1.7	48.0 – 54.0	[1]*** [3]**	48.7 ± 1.2	47.5 – 47.5 – 50.5	[1]*** [2]** [4]** [5]**	50.1 ± 1.2	48.5 – 52.0	[1]*** [3]**	50.5 ± 0.0	50.5 – 50.5	[1]** [3]**

♂	[1] Azores (n = 7)			[2] Madeira (n = 10)			[3] Canary Islands (n = 13)			[4] Morocco (n = 14)			[5] Portugal (n = 5)		
	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from
P1	50.9 ± 1.5	49.0 – 53.0	[2]** [3]*** [4]*	48.8 ± 1.9	46.5 – 52.5	[1]**	47.7 ± 1.3	46.0 – 50.0	[1]*** [4]** [5]*	49.3 ± 1.1	47.0 – 51.0	[1]* [3]*	49.5 ± 0.0	49.5 49.5	[3]*
S1	49.2 ± 1.5	47.0 – 51.0	[2]** [3]*** [4]**	47.3 ± 1.3	45.5 – 50.5	[1]**	46.6 ± 0.9	45.0 – 48.5	[1]*** [4]* [5]**	47.5 ± 1.3	45.0 – 49.0	[1]** [3]*	48.5 ± 0.0	48.5 – 48.5	[3]**
Tarsus	20.5 ± 0.8	19.0 – 21.4	[2]*	19.8 ± 1.0	17.6 – 20.9	[1]*	19.9 ± 0.5	19.1 – 20.5	–	19.9 ± 0.6	18.8 – 20.9	–	19.9 ± 0.0	19.9 – 19.9	–
NaLoSpi	7.5 ± 0.3	7.1 – 8.1	[4]*	7.4 ± 0.5	6.6 – 8.5	–	7.7 ± 0.3	7.3 – 8.1	[4]** [5]*	7.1 ± 0.4	6.6 – 8.1	[1]* [3]**	7.2 ± 0.0	7.2 – 7.2	[3]*
Bill length	16.5 ± 0.6	15.9 – 17.3	–	16.7 ± 0.7	15.4 – 17.5	–	16.8 – 0.6	15.9 – 17.7	[4]*	16.3 ± 0.6	15.6 – 17.7	[3]*	16.8 ± 0.0	16.8 – 16.8	–
Bill heigt	3.6 ± 0.4	2.9 – 4.0	[5]**	3.5 ± 0.1	3.3 – 3.7	[5]**	3.7 ± 0.4	3.3 – 4.7	[5]*	3.5 ± 0.2	3.2 – 3.8	[5]***	4.0 ± 0.0	4. 0 – 4. 0	[1]** [2]** [3]* [4]***
Bill width	5.1 ± 0.5	4.4 – 5.9	[5]***	5.2 ± 0.3	4.8 – 5.7	[4]*	5.2 ± 0.5	4.6 – 5.9	[5]***	4.8 ± 0.3	4.2 ± 5.3	[2]* [5]***	6.2 ± 0.0	6.2 – 6.2	[1]*** [2]*** [3]*** [4]***
Foot in	24.6 ± 0.8	24.0 – 26.0	–	23.4 ± 0.6	23.0 – 24.5	–	23.3 ± 1.9	20.0 – 25.0	–	24.1 ± 1.4	20.5 – 25.0	–	–	–	–
Foot mid	30.0 ± 0.6	29.0 – 31.0	–	29.3 ± 0.7	28.0 – 30.0	–	29.0 ± 2.1	26.0 – 31.0	–	29.6 ± 1.5	26.5 – 31.5	–	–	–	–
Foot out	25.4 ± 0.5	25.0 – 26.0	–	24.4 ± 0.5	24.0 – 25.0	–	24.2 ± 1.9	21.0 – 26.0	–	24.9 ± 1.4	21.5 – 26.0	–	–	–	–

♀	[1] Azores (n = 14)			[2] Madeira (n = 8)			[3] Canary Islands (n = 11)			[4] Morocco (n = 13)			[5] Portugal (n = 3)		
	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from
Weight	17.8 ± 2.0	13.7 – 20.6	[2]***	15.3 ± 1.5	13.1 – 17.3	[1]*** [3]*** [4]*** [5]*	17.9 ± 1.5	15.4 – 19.5	[2]***	18.3 ± 0.9	16.6 – 19.8	[2]***	17.5 ± 1.4	15.9 – 18.5	[2]*
Wing	72.6 ± 2.1	68.0 – 75.0	[2]***	69.1 ± 3.1	62.0 – 71.5	[1]*** [3]* [4]*** [5]*	71.2 ± 1.8	67.5 – 74.0	[2]* [4]**	73.5 ± 2.0	70.5 – 77.0	[2]*** [3]**	72.2 ± 1.6	71.0 – 74.0	[2]*
P9	48.7 ± 4.0	37.0 – 52.0	–	47.7 ± 2.1	43.5 – 50.5	[4]*	47.6 ± 1.9	44.0 – 50.0	[4]*	50.5 ± 1.8	47.5 – 54.0	[2]* [3]*	50.5	50.5	–
P8	56.2 ± 2.8	48.0 – 59.0	[2]* [3]*	54.0 ± 2.1	50.5 – 57.0	[1]* [4]**	54.4 ± 1.9	52.0 – 57.5	[1]* [4]*	56.6 ± 1.7	54.5 – 60.0	[2]** [3]*	56.0	56.0	–
P7	58.0 ± 1.4	55.0 – 60.0	[2]*** [3]***	55.6 ± 1.3	53.0 – 57.5	[1]*** [4]*	55.5 ± 1.7	53.0 – 58.0	[1]*** [4]**	57.3 ± 1.6	55.0 – 60.0	[2]* [3]**	56.0	56.0	–
P6	57.6 ± 1.2	55.0 – 60.0	[2]*** [3]*** [4]*	54.6 ± 1.6	52.0 – 56.5	[1]*** [4]*	54.8 ± 1.7	52.0 – 58.0	[1]*** [4]*	56.3 ± 1.5	54.0 – 59.0	[1]* [2]* [3]*	55.0	55.0	–
P5	54.8 ± 1.0	52.5 – 56.0	[2]*** [3]*** [4]**	52.3 ± 1.8	50.0 – 55.5	[1]***	52.2 ± 1.4	50.0 – 54.0	[1]***	53.3 ± 1.4	51.0 – 56.0	[1]**	51.5	51.5	–
P4	53.0 ± 0.8	52.0 – 54.5	[2]*** [3]*** [4]**	50.8 ± 1.5	49.5 – 53.0	[1]***	50.9 ± 1.3	48.0 – 52.5	[1]***	51.6 ± 1.4	49.0 – 54.0	[1]**	50.0	50.0	–
P3	52.4 ± 0.8	51.0 – 54.0	[2]*** [3]*** [4]**	49.9 ± 1.2	48.5 – 52.0	[1]*** [4]*	50.3 ± 1.4	47.0 – 52.0	[1]***	51.0 ± 1.4	48.5 – 53.5	[1]*** [2]*	49.5	49.5	–
P2	51.4 ± 1.0	49.5 – 53.0	[2]*** [3]**	48.7 ± 2.3	44.0 – 51.5	[1]*** [4]**	49.5 ± 1.2	47.0 – 51.0	[1]**	50.4 ± 1.3	48.0 – 52.5	[2]**	49.5	49.5	–
P1	50.0 ± 1.2	48.0 – 51.5	[2]*** [3]**	47.3 ± 1.8	44.0 – 49.0	[1]*** [4]**	48.3 ± 1.4	45.5 – 50.0	[1]**	49.3 ± 1.1	47.5 – 51.0	[2]**	47.5	47.5	–
S1	48.5 ± 1.3	45.0 – 50.5	[2]** [3]**	46.5 ± 1.7	44.0 – 49.0	[1]**	46.6 ± 1.3	44.0 – 48.0	[1]** [4]*	47.8 ± 1.2	46.0 – 49.5	[3]*	45.5	45.5	–

♀	[1] Azores (n = 14)			[2] Madeira (n = 8)			[3] Canary Islands (n = 11)			[4] Morocco (n = 13)			[5] Portugal (n = 3)		
	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from	mean ± s.d.	range	Signif. from
Tarsus	20.7 ± 0.7	19.4 – 22.0	[2]*** [4]*	19.0 ± 0.9	17.7 – 20.3	[1]*** [3]*** [4]** [5]**	20.3 ± 0.8	18.9 – 21.3	[2]***	20.0 ± 0.6	18.5 – 20.7	[1]* [2]**	20.6 ± 0.8	20.0 – 21.1	[2]**
NaLoSpi	7.4 ± 0.3	6.7 – 8.0	[4]**	7.3 ± 0.3	6.9 – 7.9	[4]*	7.4 ± 0.6	6.7 – 8.4	[4]**	7.0 ± 0.3	6.4 – 7.4	[1]** [2]* [3]**	7.2 ± 0.0	7.2 – 7.2	–
Bill length	16.8 ± 0.7	15.5 – 18.2	[4]**	16.8 ± 0.7	15.9 – 17.9	[4]**	16.4 ± 0.5	15.5 – 16.9	–	15.9 ± 0.5	15.0 – 16.7	[1]** [2]** [5]*	17.1 ± 2.2	15.5 – 18.6	[4]*
Bill heigt	3.6 ± 0.2	3.2 – 3.8	–	3.5 ± 0.2	3.3 – 3.7	–	3.6 ± 0.3	3.2 – 4.0	–	3.5 ± 0.2	3.1 – 3.8	–	3.7 ± 0.3	3.5 – 3.9	–
Bill width	5.0 ± 0.3	4.3 – 5.4	[3]**	5.0 ± 0.4	4.6 – 5.6	–	5.4 ± 0.4	4.6 – 6.0	[1]**	5.1 ± 0.2	4.8 – 5.4	–	5.3 ± 0.0	5.2 – 5.3	–
Foot in	24.5 ± 1.3	22.0 – 26.0	–	23.6 ± 0.5	23.0 – 24.0	–	24.4 ± 1.4	21.5 – 25.0	–	23.6 ± 1.1	22.0 – 25.5	–	23.0	23.0	–
Foot mid	30.3 ± 1.9	27.0 – 33.0	–	29.6 ± 1.0	28.5 – 31.0	–	30.4 ± 1.7	27.0 – 31.5	–	28.9 ± 1.3	27.0 – 31.0	–	28.0	28.0	–
Foot out	25.5 ± 1.5	23.0 – 27.0	–	24.4 ± 0.6	23.5 – 25.0	–	25.4 ± 1.7	22.0 – 26.5	–	24.8 ± 0.7	24.0 – 26.0	–	23.0	23.0	–

Appendix J Photographic documentation of island taxa covered in this thesis

European robin (*Erithacus rubecula*)

E. r. rubecula, Portugal

E. r. superbus, Tenerife (Canary Islands)

Island canary (*Serinus canarius*)

S. canarius, St. Miguel (Azores)

S. canarius, La Gomera (Canary Islands)

Goldcrest (*Regulus regulus*) and Firecrest (*Regulus ignicapillus*)

R. r. azoricus, Terceira (Azores)

R. r. teneriffae La Gomera (Canary Islands)

R. madeirensis, Madeira

Blue tit (*Parus caeruleus / teneriffae*)

P. c. oglistrae, Portugal

P. t. ultramarinus, Fuerteventura (Canary Islands)

P. t. teneriffae, La Gomera (Canary Islands)

P. t. ombriosus, El Hierro (Canary Islands)

Sardinian warbler (*Sylvia melanocephala*), Blackcap (*S. atricapilla*), Spectacled warbler (*S. conspicillata*)

S. m. leucogastra, El Hierro (Canary Islands)

S. m. melanocephala, Morocco

S. a. heineken, Madeira

S. c. orbitalis, Lanzarote (Canary Islands)

Appendix K Photographic documentation of taking blood samples in passerine birds

Handling of bird to access the *Vena jugularis* (Photo: D. Guicking)

Location of the *Vena jugularis* on the underside of the wing (Photo: D. Guicking)

Obtaining blood sample after punctuating the *Vena jugularis* with a sterile syringe (Photo: D. Guicking)

Hiermit versichere ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Datum	Christian Dietzen