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Phylogeography of island canary (*Serinus canaria*) populations

Abstract Island canaries (*Serinus canaria*) are characterised as a species living exclusively on North Atlantic islands, mainly on the Azores, Madeira and Canary Islands. Although they are very common in their habitats, their behaviour and breeding system has only recently been studied systematically. To advance the understanding of their ecology and to see if the rather isolated archipelagos are already promoting a genetic differentiation, we investigated their phylogeographic relationship as revealed by mtDNA sequences of the cytochrome *b* gene and investigated whether this measure corresponds to morphological characteristics within the islands. Genetic distances were very low throughout the distribution range of the species. Although the variation of genetic distances within the population of Pico (Azores) was larger than that on Madeira and Canary Islands, the genetic distances between island populations were very low throughout which prevented a clear phylogeographic differentiation. Moreover, morphological measurements did not reveal a consistent pattern to reliably separate the populations, although the measures of beak length and body weight revealed a clear island-specific differentiation. These data lead to the assumption that the colonisation of the Atlantic islands by the canaries occurred very recently, while there is no persisting gene flow between the populations.

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Introduction

The genetic structure of a population reflects underlying determinants, such as gene flow, age structure and mating systems (e.g. 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 (for review, see Petrie and Kempenaers 1998; Wink and 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. If we assume that the relationship between population size and area of landmass is a continuous scale, then we are allowed 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 Galápagos 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 and Leitner 1998; Leitner et al. 2003). Moreover, in a parentage analysis on island canaries, we did not find evidence for EPP, which we believe can 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 Ilhéu Chão (Madeiran Archipelago) which is only 0.5 km^2 in size (Fig. 1). Our aim 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 (e.g. Arnaiz-Villena et al. 1998, 1999, 2001; Salzburger et al. 2002a, b; Weibel and Moore 2002; Marks et al. 2002; Ericson et al. 2002; Irestedt et al. 2002; Ericson and Johansson 2003; Dietzen et al. 2003; Päckert et al. 2005).

Therefore, in the island canary we were interested: (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 colonisation 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.

Materials 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 Ilhéu Chão (32°35′N, 16°32′W), both Madeiran Archipelago, from 1995 to 1999, as well as on Pico, Azores (Candelária: 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 (Table 1). 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.L.) 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 in Leisler et al. (1997), Broders et al. (2003) and 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

Fig. 1 The Macaronesian region comprises the archipelagos of the Azores, Madeira, the Selvagens Islands, the Canary Islands and Cabo Verde Islands. Populations of island canaries (*Serinus canaria*) only occur on the Azores, Madeira and Canary Islands. The Azores have a total landmass of about 2,300 km^2, Madeira 794 km^2 and the Canary Islands 7,273 km^2. Islands from which samples have been collected are *underlined*
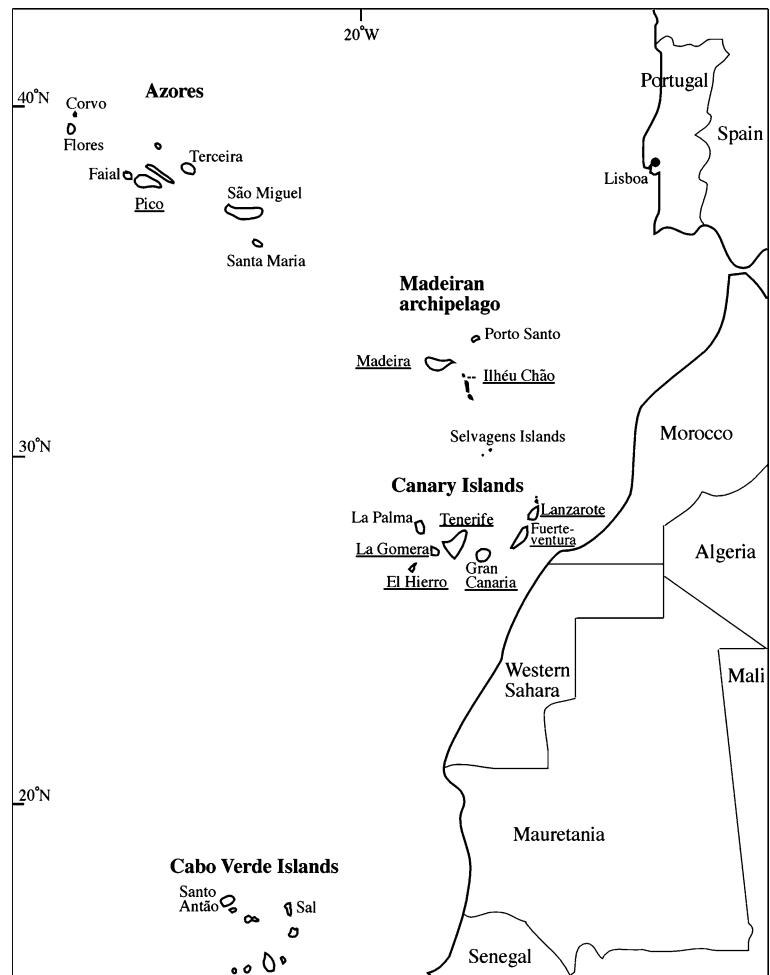
Table 1 Sampling locations for island canaries (*Serinus canaria*) and number of samples that were used for genetic and morphological analysis

Region	Location	Latitude/longitude	Number of birds genetic analysis	Number of birds morphological analysis
Pico/Azores	Candelária	38°28′N, 28°31′W	14	26
	Serra	38°30′N, 28°20′W	–	2
Ilhéu Chão	Ilhéu Chão	32°35′N, 16°32′W	30	205
Madeira	Ponta do Pargo	32°49′N, 17°17′W	5	12
	Santana	32°48′N, 16°54′W	6	16
Canary Islands	El Hierro	27°42′N, 18°01′W	2	–
	La Gomera	28°04′N, 17°15′W	2	–
	Tenerife	28°20′N, 16°31′W	1	–
	Gran Canaria	28°07′N, 15°37′W	2	–
	Fuerteventura	28°16′N, 14°01′W	2	–
	Lanzarote	29°02′N, 13°38′W	1	–

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$_4$Ac (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 and 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 and Rozas 1999).

An appropriate substitution model for the molecular dataset was estimated via likelihood ratio test with Modeltest 3.04 (Posada and Crandall 1998). The selected model was the Tamura–Nei model (TRN + G; Tamura and Nei 1993). Likelihood settings were as follows: empirical base frequencies $\pi A = 0.2927$, $\pi C = 0.3307$, $\pi G = 0.1316$, $\pi T = 0.2450$; 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, we compared genetic distance values from individuals on the Azores (Pico) ($n = 14$), Madeira ($n = 11$), Ilhéu 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 Ilhéu Chão ($n = 205$) (Table 1). 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.

Results

Genetic distances

A complete fragment of 1,000 nucleotides of the mitochondrial cytochrome *b* gene was sequenced from 65 island canaries. Sequences have been submitted to Genbank (accession numbers AY914098–AY914162). The island canary dataset comprised 22 different haplotypes (haplotype diversity, $h = 0.678$; nucleotide diversity, $\pi = 0.00190$) with 38 variable sites of which 30 were parsimony informative. The nucleotide and haplotype diversity was highest within the Azorean birds ($\pi = 0.00420$, $h = 0.94505$), the highest theta value was also found there ($\theta = 0.00775$). The lowest diversity values were found on Madeira (Table 2). 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 3 for mean values). In some cases genetic distances of individual birds within a population were even larger than from individuals between different populations. However, we found significant differences between populations 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 Ilhéu 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 $0.37 \pm 0.04\%$.

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 3). 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).

Phylogenetic analysis

As genetic distances were rather small, no tree building algorithm revealed any conclusive phylogenetic or phylogeographic patterns. Variable characters and

Table 2 DNA polymorphism in the studied populations of island canary

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	< 0.05	14
Madeira[a]	0.00119	0.50366	0.00432	11	−2.36028	< 0.01	41
Canary Islands	0.00135	0.75556	0.00181	5	−1.03527	> 0.10	10

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 [a] Samples from Ilhéu Chão are included in Madeira for this analysis

Table 3 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

	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**

Genetic distances are presented as mean values with standard deviations. The diagonal (*bold numbers*) represents mean values within the respective populations

haplotypes in the data set are documented in Table 4. The minimum spanning network showed a clearly star-like topology connecting all 22 haplotypes (Fig. 2). 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 (Fig. 2, hatched circle). This suggests a recent range expansion which is also

Table 4 Variable characters and frequency in the cytochrome *b* haplotypes in three island canary populations
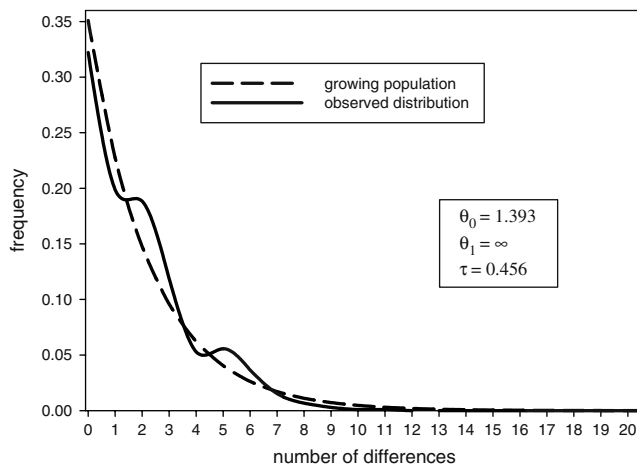
	Haplotype (N)	Accession number	Frequency MD	AZ	CI	Variable sites `1111112 3334444445 5555556678 99999900` `6671344789 0361788990 0144691255 23488900` `5647716781 5820302571 5109121610 62819557` (last group prefixed `11`)
02_TF	A (37)	AY914098	0.71	0.21	0.50	`ATCCTCACAA CTTCTCCCCC CACCACTCTA ATTCTTCT`
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
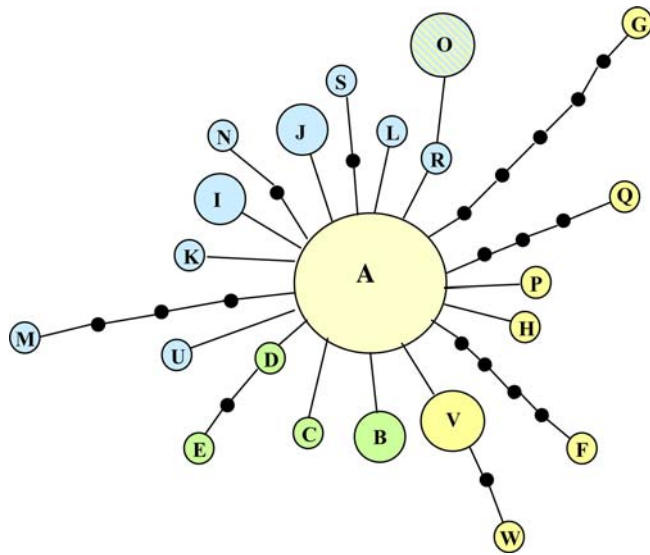
Fig. 2 Minimum spanning network for 22 haplotypes (A–S, U–W, see Table 4 for reference) of 65 samples from island canaries in Macaronesia based on 1,000 bp of the mitochondrial cytochrome *b* gene. Haplotype frequency is indicated by *dot size* and origin of haplotypes is described by different colours (*yellow* Azores; *blue* Madeira, including Ilhéu Chão; *green* Canary Islands). Only haplotypes *A* and *O* are shared between different archipelagos
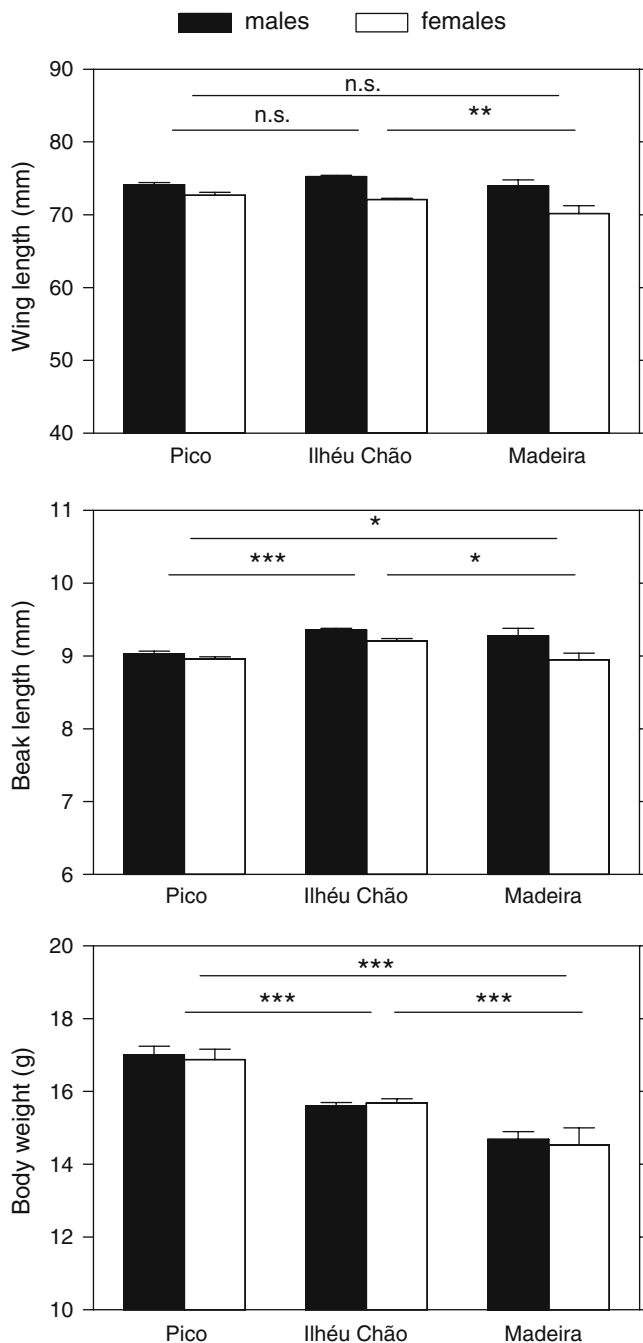
Morphological data

We analysed morphological data of a total of 261 birds from Pico/Azores, Ilhéu Chão and Madeira and found significant differences between these populations in all three measurements (Fig. 4).

1 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 Ilhéu Chão ($P = 0.008$).

2 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 Ilhéu Chão ($P < 0.0001$) and Madeira ($P = 0.014$).

3 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 Ilhéu Chão and Madeira populations ($P < 0.0001$ for all comparisons).

Fig. 3 Pairwise mismatch distribution of 65 samples from island canaries' 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

confirmed by significantly negative values of Tajima's *D* in all populations (Table 2).

From the pairwise mismatch distribution no population structuring can be detected (Fig. 3). 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 ($P < 0.01$).

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 3) could indicate:

1 The colonisation of the Atlantic islands by island canaries did occur rather recently. A sudden range expansion is also corroborated by the demographic history (Fig. 3), Tajima's *D* value and the topology of the minimum spanning network (Fig. 2). If we assume 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 our 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.

2 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

Fig. 4 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

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 (Bannerman and Bannerman 1966), which, according to our data, 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 (Dietzen et al. unpublished). 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* sp.) which revealed a strong substructuring with highly distinct taxa even on neighbouring islands (Marshall and Baker 1999; Dietzen et al. 2003; Kvist et al. 2005; Päckert et al. 2005).

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 and Leitner 1998). On the other hand, there are also reports of larger scale movements. Bannerman and Bannerman (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 and Scheer 1971). These observations indicate at least a potential ability of canaries to travel

confinement of most of the haplotypes to certain islands or island groups (private alleles) 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

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 and Baker 1999; Dietzen et al. 2003).

Concerning the mating system, island canaries are socially monogamous, as we recently found no evidence for EPP in the population of the only 0.5-km^2 island, Ilhéu Chão (Voigt et al. 2003). At the nearest point, Ilhéu 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 Galápagos 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 our island canary study. Here, the intra- and inter-population genetic distances overlap and a distinct phylogeographic pattern cannot be recovered.

However, in our populations, we could also observe subtle yet recognisable morphological differences between islands. 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

and Short 1994; Sjögren 2001). Anyway, the subtle morphological differences in the canaries do not correspond to the genetic profile in our 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 that we found in our 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 and 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 and Baker 1994) and the crests and kinglets (Päckert et al. 2003, 2005). 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.

Zusammenfassung

Phylogeographie der Populationen des Kanarengirlitz (*Serinus canaria*)

Der Kanarengirlitz (*Serinus canaria*) stellt eine Art dar, die hauptsächlich auf den nordatlantischen Inseln (Azoren, Madeira und Kanarische Inseln) heimisch ist. Obwohl er dort in seinem Lebensraum recht häufig vorkommt, wird sein Verhalten und seine Brutbiologie erst seit kurzem systematisch untersucht. Zum besseren Verständnis seiner Ökologie und im Hinblick auf eine mögliche genetische Differenzierung der eher isolierten Archipele untersuchten wir die phylogeographische Beziehung mit Hilfe der mitochondrialen DNA Sequenzen des Cytochrom b Gens und verglichen diese Befunde mit morphologischen Merkmalen. Die genet-

ischen Distanzen waren innerhalb des Verbreitungsgebietes der Art recht niedrig. Obwohl die genetischen Distanzen innerhalb der Population von Pico (Azoren) größer als die von Madeira und den Kanarischen Inseln waren, zeigten die allgemein geringen Werte keine klare phylogeographische Differenzierung. Allerdings konnten geographisch eingeschränkt verbreitete, inselgruppen-spezifische Allele gefunden werden. Die morphologischen Merkmale ergaben kein einheitliches Muster, um die Populationen in einer verlässlichen Weise zu trennen, jedoch ergaben sich bei der Schnabellänge und dem Körpergewicht klare inselspezifische Differenzierungen. Diese Daten führen zu der Annahme, dass die Besiedlung der atlantischen Inseln durch den Kanarengirlitz erst vor relativ kurzer Zeit stattfand wobei kein anhaltender Genfluss zwischen den Populationen besteht.

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