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Return flight to the Canary Islands – The key role of peripheral populations of Afrocanarian blue tits (Aves: *Cyanistes teneriffae*) in multi-gene reconstructions of colonization pathways

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ABSTRACT

Afrocanarian blue tits (*Cyanistes teneriffae*) have a scattered distribution on the Canary Islands and on the North African continent. To date, the Canary Islands have been considered the species' main Pleistocene evolutionary center, but their colonization pathways remain uncertain. We set out to reconstruct a dated multi-gene phylogeny and ancestral ranges for *Cyanistes* tit species including the currently unstudied, peripheral Libyan population of *C. t. cyrenaicae*. In all reconstructions the most easterly and westerly peripheral populations (in Libya and on La Palma) represented basal offshoots of *C. teneriffae*. These two peripheral populations shared all four major indels and differed in this respect from all other members of the Afrocanarian core group. The basal split of Afrocanarian blue tits from their European relatives was dated to the early Pliocene. The two ancestral area reconstructions were contradictory and suggested either a Canarian or a North African origin of *C. teneriffae* – but unambiguously ruled out a continental European ancestral range. We conclude that the peripheral populations of *C. teneriffae* represent relic lineages of a first faunal interchange, presumably downstream colonization from North Africa to the Canary Islands. Subsequent eastward stepping-stone colonization within the Canarian Archipelago culminated in a very recent late (possibly even post-) Pleistocene back-colonization from the Canary Islands to North Africa.

1. Introduction

Peripheral populations at the margins of a species' continental range have attracted the interest of ecologists and evolutionary biologists because they provide an opportunity to study the ecological and evolutionary consequences of colonization pathways. Peripheral populations are often fragmented and when separated by larger distances often show remarkable genetic differentiation from central, core populations (Schwartz et al., 2003; Kvist et al., 2007; Lehtonen et al., 2009; Hardie and Hutchings, 2010; Piñeiro et al., 2011; Pandey and Rajora, 2012a,b). This pattern is not restricted to genetic markers, but can also be observed for morphological (Cassel-Lundhagen et al., 2009), physiological (Garland and Adolph, 1991; Broggi et al., 2005) and behavioral traits such as the song repertoires observed in willow tits (*Poecile montana*; Quaisser and Eck, 2003). Patterns of genetic differentiation often become more pronounced when island populations at the periphery of a species' distribution are considered (Burg et al., 2006; Päckert et al., 2006; Tomozawa and Suzuki, 2008; Barrientos et al., 2009) and a similar pattern is found between central and peripheral islands (Illera et al., 2007; Warren et al., 2012).

The Afrocanarian blue tit *Cyanistes teneriffae* is one such extreme example having highly differentiated island populations in the West but also scattered and isolated continental populations to the East, in North Africa (Cramp and Perrins, 1993; del Hoyo et al., 2007). A recent molecular study by Illera et al. (2011) has shed some light on the evolutionary history of *Cyanistes* as a whole. According to their results the extant monophyletic Afrocanarian blue tit lineage originated from a Pleistocene refuge and center of diversification on the Canarian archipelago that may even have provided a source population for a back-colonization to the North African continent. Nevertheless, the ancestral continental area of *C. teneriffae* remained obscure so far, and the species' radiation on the Canary Islands is believed to have started from founder populations either on Tenerife (Kvist et al., 2005) or on La Palma (Illera

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et al., 2011). Both theories seem to contradict the continent-to-island stepping-stone model (Cox et al., 2010; Emerson, 2002; Sanmartín et al., 2008).

Any extinction of lineages and incomplete sampling of extant taxa can obscure phylogenetic conclusions regarding pathways of colonization and arrival times (Emerson et al., 2000; Johnson, 2001; Omland et al., 1999). In this context, the isolated Afrocanarian blue tit populations from Libya (*C. t. cyrenaicae*) are of particular interest. Given their extreme separation from populations in the West (Morocco, Algeria, Tunisia: *C. t. ultramarinus*) and in the East (Levant: *C. t. satunini*) the Libyan populations might have played a key role during faunal interchange among the European/Near East and North African populations on the one hand, and during the colonization of the Canary Islands on the other.

In this paper we provide a multi-gene phylogeny based on three mitochondrial and two nuclear markers for 17 out of 22 currently accepted *Cyanistes* blue and azure tit taxa (sensu Dickinson, 2003) inclusive of some genetically distinct island lineages. We specifically aim to explore the speciation processes and colonization pathways within Afrocanarian blue tits and to deduce their ancestral areas via (1) estimates of the time split ages among distinctive genetic lineages and (2) reconstruction of ancestral ranges using likelihood and parsimony analyses.

2. Materials and methods

2.1. Phylogentic reconstruction

For phylogenetic reconstructions we sequenced three mitochondrial and two nuclear markers (see below) for 17 (out of 22) subspecies of the three currently accepted *Cyanistes* species. We used 35 samples representative of each of the mitochondrial and nuclear lineages from our previous studies (inclusive of newly analyzed Libyan samples) and completed individual sequence data sets in order to produce multigene phylogenetic reconstructions (Table 1; for primers and PCR settings see Päckert et al., 2012b). DNA was extracted from blood and tissue samples in a chloroform–isoamyl isolation or with a peqLab tissue/blood kit according to the manufacturer's instructions.

A fragment of domain 1 of the mitochondrial control region was amplified using standard primers L16700 and H636 (Kvist et al., 1999) and the anonymous marker 12,884 was amplified with primers 12,884-forw and 12,884-rev (Backström et al., 2008). Amplification profiles for both markers were performed according to Illera et al. (2011). Cytochrome-*b* was amplified in a double-stranded PCR using the primer combination L14841-Cyt*b* and H15917-Cyt*b*. A fragment of the 16S ribosomal RNA gene was amplified using the primer combination 16S500 and 16Sa, and amplification of fibrinogen intron 7 was performed with the primer combination Fib-17S-L and Fib-17S-U.

PCR products were purified by adding 0.1 μl ExoSap-IT solution in 4 μl distilled water to each sample (cycling program: 37 °C for 30 min and 94 °C for 15 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 manufacturers' instructions. Cycle sequencing products were purified by salt/ethanol precipitation or by using Sephadex (GE Healthcare, München, Germany) and sequenced in both directions on an ABI 3130xl DNA sequencer. The sequences were aligned by ClustalW using MEGA 3.1 (Tamura et al., 2011) and slightly adjusted by eye.

New control region and nuclear 12,884 sequences were added to the data set of Illera et al. (2011) and new cytochrome-*b* se-

Table 1
Samples used for sequencing analysis; 16S rRNA (16S) and fibrinogen intron 7 (fib7) sequenced only for a number of samples used for multigene reconstructions; KC202302–KC202410 were newly sequenced for this study.

Sample	Species	Subspecies	Country	Locality	cyt*b*	CR	nuc12884	16S	Fib7
MTD487	*C. caeruleus*	*caeruleus*	Germany	Dresden	JF828081	JF828052	KC202409	KC202330	KC202392
ST231Gl	*C. caeruleus*	*obscurus*	UK	Glasgow	KC202350	AY137803	JF755032	–	KC202382
NHMC80.4.167.4	*C. caeruleus*	*calamensis*	Greece	Crete	KC202361	JF828056	–	–	–
NHMC80.4.167.9	*C. caeruleus*	*calamensis*	Greece	Crete	JF828080	JF828058	KC202410	KC202329	KC202379
ST110	*C. caeruleus*	*ogliastrae*	France	Corsica	KC202351	AY267066	KC202402	–	KC202380
ST115	*C. caeruleus*	*ogliastrae*	France	Corsica	KC202352	AY267050	KC202403	–	KC202381
ST594	*C. caeruleus*	*raddei*	Iran	Noor	KC202353	EF523800	KC202404	KC202323	KC202383
ST597	*C. caeruleus*	*raddei*	Iran	Noor	KC202354	EF523803	JF755049	–	KC202384
ST936	*C. caeruleus*	*satunini*	Jordan	Dibbeen	KC202359	EF523808	JF755042	KC202327	KC202389
ST937	*C. caeruleus*	*satunini*	Jordan	Dibbeen	KC202360	EF523809	KC202406	–	KC202390
MAR123	*C. cyanus*	*cyanus*	Russia	W of Ural Mts	KC202332	KC202305	KC202393	KC202308	–
MAR1600	*C. cyanus*	*flavipectus*	Kyrgyzstan	Sari-Tshelek	KC202336	DQ483103	JF755013	KC202313	KC202368
MAR1642	*C. cyanus*	*tianshanicus*	Kyrgyzstan	Issyk Kul	KC202337	DQ483105	JF755015	KC202314	KC202369
MAR342	*C. cyanus*	*yenisseensis*	Russia	Rep. Altai	KC202334	–	KC202394	KC202310	KC202364
MAR4016	*C. cyanus*	*yenisseensis*	Mongolia		KC202345	DQ483108	JF755017	–	KC202374
MAR7991	*C. teneriffae*	*cyrenaicae*	Libya	Close to Cyrene	KC202346	KC202302	KC202398	KC202320	KC202375
MAR7992	*C. teneriffae*	*cyrenaicae*	Libya	Close to Cyrene	KC202347	KC202301	KC202399	–	KC202376
MAR7993	*C. teneriffae*	*cyrenaicae*	Libya	Close to Cyrene	KC202348	KC202304	KC202400	KC202321	–
MAR8040	*C. teneriffae*	*cyrenaicae*	Libya	Close to Cyrene	KC202349	KC202303	KC202401	–	–
MAR1163	*C. teneriffae*	*degener*	Spain	Fuerteventura	JF828078	AY588281	KC202395	KC202311	KC202365
MAR1166	*C. teneriffae*	*degener*	Spain	Lanzarote	JF828088	AY588282	JF755127	KC202312	KC202366
ST1642	*C. teneriffae*	*degener*	Spain	Fuerteventura	KC202307	JF755284	JF755117	KC202328	KC202391
CH7260	*C. teneriffae*	*hedwigi*	Spain	Gran Canaria	KC202363	KC202306	KC202408	KC202322	KC202378
MAR3504	*C. teneriffae*	*ombriosus*	Spain	El Hierro	KC202338	AY538231	JF755206	–	–
MAR3511	*C. teneriffae*	*ombriosus*	Spain	El Hierro	KC202339	AY538232	JF755207	KC202315	KC202370
MAR3519	*C. teneriffae*	*palmensis*	Spain	La Palma	KC202340	AY538223	KC202396	KC202316	KC202371
MAR3522	*C. teneriffae*	*teneriffae*	Spain	Tenerife	KC202342	AY538210	JF755143	KC202317	KC202372
MAR3526	*C. teneriffae*	*teneriffae*	Spain	Tenerife	KC202341	AY538211	JF755144	KC202318	–
MAR3527	*C. teneriffae*	*teneriffae*	Spain	La Gomera	KC202343	AY538233	KC202397	KC202319	KC202373
MAR3528	*C. teneriffae*	*teneriffae*	Spain	La Gomera	KC202344	AY538234	JF755175	–	–
MAR142	*C. teneriffae*	*ultramarinus*	Morocco	NW of Meknes	KC202333	JF755387	–	KC202309	–
ST901	*C. teneriffae*	*ultramarinus*	Morocco	Ceuta	KC202357	JF755349	JF755102	–	KC202387
ST902	*C. teneriffae*	*ultramarinus*	Morocco	Ceuta	KC202358	JF755350	JF755103	KC202326	KC202388
ST668	*C. teneriffae*	*ultramarinus*	Italy	Pantelleria	KC202355	JF755362	JF755090	KC202324	KC202385
ST669	*C. teneriffae*	*ultramarinus*	Italy	Pantelleria	KC202356	JF755363	KC202405	KC202325	KC202386

quences were compared with those of Dietzen et al. (2008). Haplotype networks were created with TCS v1.21 (Clement et al., 2000).

The appropriate substitution model for each of the five sequence data sets was estimated using MrModelTest (Nylander, 2004). Maximum likelihood (ML) phylogenetic trees were reconstructed using raxML7.2.8 (Stamatakis, 2006; Silvestro and Michalak, 2010; 1000 thorough bootstrap replicates). Bayesian inference of phylogeny was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) using the Metropolis-coupled Markov chain Monte Carlo algorithm with two parallel runs, each with one cold and three heated chains each run for 30,000,000 generations with every 100th generation sampled (burn-in: 9000). Bayesian and maximum likelihood analyses were performed by partitioning the concatenated sequence data according to the different gene fragments (including partitioning by codon position for cyt*b*). For each partition, basic model settings were applied and the GTR+I+Γ was estimated the best fit model for four markers (cyt*b* codon positions 1, 2 and 3, CR, 16S rRNA, 12,884) "lset nst = 6 rates = invgamma". Only for fib7 HKY+Γ was estimated the best fit model "lset nst = 2 rates = gamma". Because raxML offers only a limited choice of model settings, the GTR+I+Γ model was applied across all partitions in likelihood reconstructions.

In runs with MrBayes we allowed the overall rate to vary between partitions by setting the priors ⟨ratepr = variable⟩ and model parameters such as gamma shape and proportion of invariable sites unlinked across partitions, so that for each partition a separate set of parameters was estimated. For comparison, phylogenetic reconstructions were also carried out for cytochrome-*b* data alone.

2.2. Molecular dating

Divergence times among *Cyanistes* clades were estimated with BEAST 1.4.8 (Drummond and Rambaut, 2007). BEAST was run with 30,000,000 generations with the "auto optimize" option activated and Yule process prior implemented.

We performed two independent runs with an extended cytochrome-*b* data set including sequence data from Gill et al. (2005; GenBank accession Nos.: AF347937–347963 and AY308718–308735). The first run was performed by applying an empirical fixed substitution rate of 0.0105 (Weir and Schluter, 2008). In the second run no fixed rate was assigned *a priori* to the data but four nodes of the outgroup clades were calibrated using fixed node ages that should correspond to paleogeographic events such as emergence of volcanic islands and opening/closure of land bridges (Table A2; cf. Päckert et al., 2012b).

The multigene tree was calibrated at two outgroup nodes, outgroup sequences of other tit species from *Parus*, *Periparus* and *Poecile* were taken from Päckert et al. (2012b). The concatenated sequence data set was partitioned by gene and codon position (cyt*b*) with best fit substitution models applied separately to each of the seven partitions (see above) and a second independent run with BEAST was performed with an unpartitioned data set under the same settings. A third independent run with BEAST was performed with fixed node ages applied to the same two outgroup nodes and to three further *Cyanistes* ingroup nodes (volcanic ages of La Palma and El Hierro to the respective lineage splits and the beginning of the Messinian Crisis to the basal node separating the Afrocanarian lineage from its continental Eurasian sister clade; Table A2).

Linearized consensus trees including posterior probabilities were inferred from the tree output files (concatenated sequence data sets) using TreeAnnotator v. 1.4.8 (as implemented in the BEAST package) with the burn-in parameter set to 9000 and node heights set to "mean heights". Time estimates of lineage splits and mean substitution rates were inferred from the log output files using Tracer v1.4 software (Rambaut and Drummond, 2007).

Table 2
Stepmatrix model applied to maximum parsimony ancestral range reconstructions with Mesquite; costs of dispersal among neighboring regions set to 1 and costs were doubled with each region to be crossed.

Region	E. Pal.	W. Pal.	Med. Isls.	N. Africa	Canary Isls.
East Palearctic	0	1	2	2	4
West Palearctic	1	0	1	1	2
Mediterranean Islands	2	1	0	1	2
North Africa	2	1	1	0	1
Canary Islands	4	2	2	1	0

2.3. Historical biogeography

Ancestral ranges of clades were inferred using the ancestral state reconstruction package Mesquite 2.5 (Maddison and Maddison, 2008). The Bayesian consensus tree (partitioned by gene and codon position) was loaded as the phylogenetic backbone in a Mesquite file. Only one specimen (=concatenated sequence) per taxon or island lineage was retained in the stored tree. Geographic regions were coded in a character matrix (Table 2).

There is no evidence for a clear and well supported outgroup of *Cyanistes* tits (in the sense of a sister taxon) even when comprehensive Paridae data sets are considered (Gill et al., 2005; Tietze and Borthakur, 2012). However, in the latter phylogenies, *Parus* appeared as a weakly supported sister group to *Cyanistes* and at least some of our own single- and multilocus reconstructions strongly supported this relationship. We therefore chose *P. major* as a closely related outgroup and *Poecile montana* as a more distantly related outgroup for input tree reconstruction in the ancestral state analysis. Because ML reconstructions do not allow for polymorphic character states in Mesquite and the two outgroup taxa were polymorphic with respect to their ranges, we ran a first ML reconstructions with the ingroup data set only. We performed a second ML analysis including the outgroups by lumping the two Palearctic regions into one and by treating the great tit *Parus major* as a uniquely Palearctic species in order to avoid multiple character states in the outgroups. Both ML reconstructions yielded largely congruent results if not stated otherwise below.

In order to account for phylogenetic uncertainty in the Bayesian consensus tree, we used the Mesquite function "trace character over trees" to examine a series of 21,000 trees from the Bayesian analysis using MrBayes (after deletion of the first 9000 trees). Likewise, the analysis takes into account whether a node is absent or whether at one node the character state is equivocal, i.e. having two states that are equally parsimonious. First we used the likelihood reconstruction method (Schluter et al., 1997; Pagel, 1999) with the Markov k-state 1 parameter model (Mk1 model) applied as implemented in Mesquite, with rate of character state change as the single parameter constrained to be equal for all state changes. Default settings of 2.0 for the likelihood decision threshold yielded highly equivocal results at several nodes of the tree. We therefore decreased the decision threshold stepwise to 1.0, 0.5 and 0.25.

Further independent runs with Mesquite ancestral ranges were inferred using the maximum parsimony (MP) reconstruction method under a user-defined stepmatrix model (Table 2). The stepmatrix model was based on the premise that faunal interchange between neighboring bioregions is common and likely to occur with an assumed minimum cost, whereas interchange between two regions becomes less likely with increasing distance. The cost of dispersal to a neighboring region was set to "1" and

costs were assumed to double with each extra regional crossing to be made (see Table 2).

All calculations were carried out under the default setting "count trees with uniquely best states". With this option a tree is counted as having a state at a node only if that state is unambiguously optimal. If two or more states are equally parsimonious, then the character state is counted as "equivocal". In ML reconstructions the decision threshold decides whether a state is considered optimal (Maddison and Maddison, 2008). In order to check ancestral character states at highly equivocal nodes in detail two alternative calculations were performed. In MP reconstructions the "count all trees with state" option considers all optimal states regardless of whether there is more than one state at the same node within the optimal set (i.e. two equally parsimonious states). In ML reconstructions "average frequencies across trees" were calculated for all states at each node (Maddison and Maddison, 2008).

3. Results

3.1. Single locus reconstructions

Strikingly, all single-locus reconstructions of four markers consistently supported a close relationship between the two outermost peripheral populations of *C. teneriffae* from La Palma and Libya, and largely separated them from all other Afrocanarian populations. The 16S rRNA sequence data set did not contain significant intraspecific phylogenetic signal in *Cyanistes* and consequently we shall not discuss this marker further. In a minimum spanning network of mitochondrial control region sequences in *C. teneriffae* the two peripheral populations from Libya and La Palma were connected to either of two European *C. caeruleus* clusters by at least 19 substitutions (Fig. 1A). Control region sequences from Libya and from La Palma differed by at least 11 substitutions from each other and cyt*b* sequences differed among the two respective clades by mean p-distance values of 3.5%. The cyt*b* haplotype network (Fig. 1B) and the cyt*b* tree for the full Paridae data set (Fig. A1) strongly supported this subdivision. Libyan *C. t. cyrenaicae* samples differed from allopatric Moroccan populations by 4.6% cyt*b* p-distances, while Moroccan samples shared one single cyt*b* haplotype with eastern Canary Island populations (Fig. 1B). Island populations from Tenerife and La Gomera shared haplotypes of all three mtDNA markers.

The two nuclear markers confirm the basal subdivision of *C. teneriffae*. The anonymous nuclear marker 12,884 showed a highly complex differentiation pattern of two alleles with different fragment lengths (369 and 382 base pairs) due to three indels. In the minimum spanning network (Fig. 2A) the peripheral cluster grouped the outermost populations from La Palma (ten5, ten6) and Libya (ten7) with all samples from El Hierro and La Gomera (Fig. 2A; ten4 and three others nested in the peripheral cluster), and three rare haplotypes found in seven samples from Morocco and Fuerteventura (Fig. 2A; box "core 2": ten3 and two derived haplotypes). The fib7 sequences showed less intrageneric variation and comprised ten nuclear haplotypes (Fig. 2B). The two haplotypes of the core group were separated by a five base pair indel from peripheral La Palma and Libyan populations, and from all remaining Eurasian *C. caeruleus* and *C. cyanus* sequences. Phylogenetic reconstructions based on the fib7 data set reflected the bifurcated topology of *Cyanistes* in an Afrocanarian clade of *C. teneriffae* (with lineages from Libya and La Palma being sequentially basal to the core group), as opposed to a continental Palearctic clade of *C. caeruleus* and *C. cyanus* (tree not shown).

3.2. Multilocus phylogeny and indels

In all multilocus reconstructions each of the three *Cyanistes* species represented a monophyletic group: Palearctic *C. caeruleus* and *C. cyanus* were sister species and a basal split separated them from

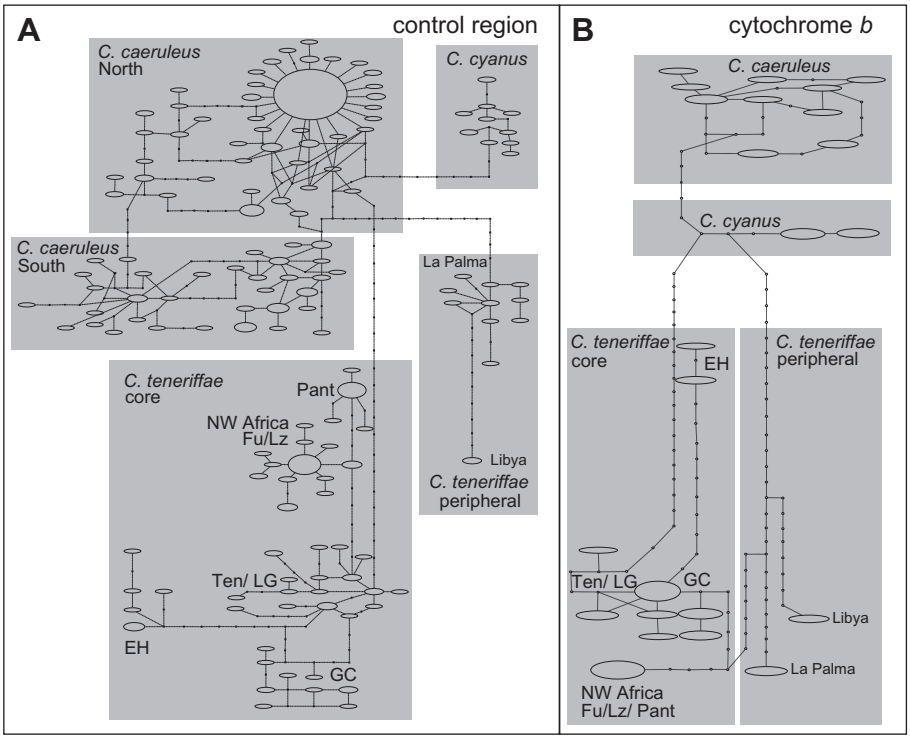

Fig. 1. Mitochondrial DNA differentiation in *Cyanistes* tits, minimum spanning networks for (A) control region sequences ($n = 110$; 504 base pairs) and (B) cytochrome-*b* sequences ($n = 54$; 594 base pairs); core island populations: Ten = Tenerife, LG = La Gomera, GC = Gran Canaria, EH = El Hierro, Fu = Fuerteventura, Lz = Lanzarote, Pant = Pantelleria.

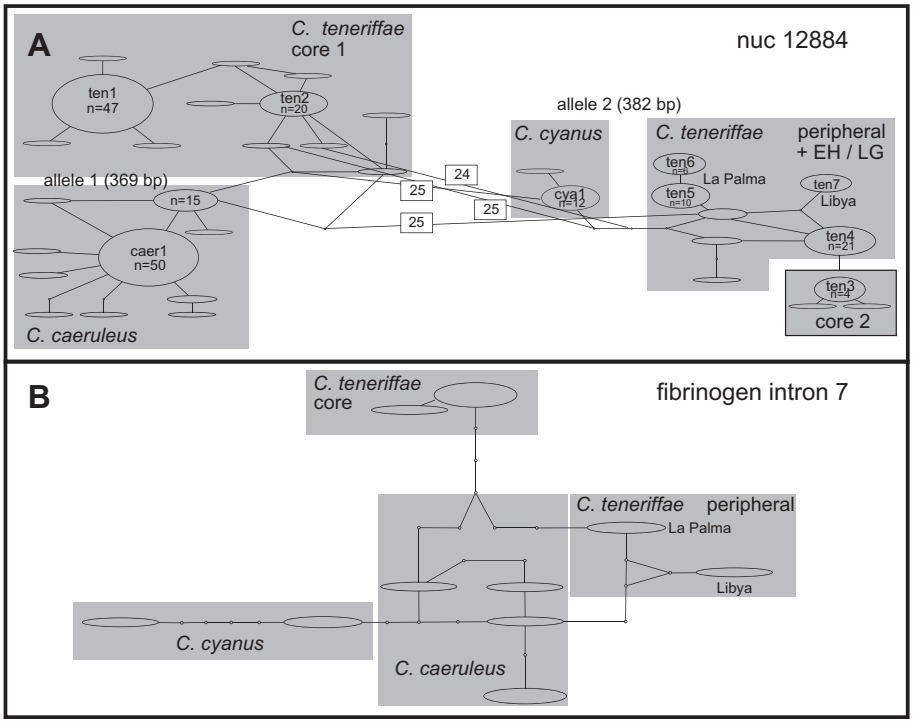

Fig. 2. Differentiation of nuclear markers in *Cyanistes* tits, minimum spanning networks; (A) two alleles of the anonymous nuclear marker 12,884 (n = 222; 385 base pairs); core 1 = samples from Tenerife, Gran Canaria, Lanzarote, Fuerteventura, North Africa; core 2 = samples with aberrant allele variation from Fuerteventura and Morocco; peripheral = along with samples from Libya and La Palma includes all samples from El Hierro and La Gomera; (B) fibrinogen intron 7 (n = 25; 615 base pairs).

the Afrocanarian *C. teneriffae* (Fig. 3). Within *C. teneriffae*, the two outermost peripheral populations from Libya and La Palma were sister to each other and represented a basal split from the strongly supported Afrocanarian core group. The peripheral lineages differed by two indels from all remaining *C. teneriffae* lineages (CR and fib7) and from the Afrocanarian core group by three indels at locus 12,884 (but shared these indels with El Hierro and La Gomera; Figs. 2A and 3). The interior topology of the core group comprised sequential splits from westernmost El Hierro eastwards to neighboring La Gomera, Tenerife, Gran Canaria and a terminal clade comprising lineages from eastern Canary Islands, Morocco and Pantelleria (Fig. 3). However, that topology received strong support only from BEAST analysis of the partitioned multilocus data set, while unpartitioned BEAST analysis and partitioned raxML analysis yielded a poor resolution of the core clade, except strong support for the terminal group (Fuerteventura, Morocco, Pantelleria; Fig. 3).

Within Eurasian blue tits, *C. caeruleus*, the island lineage from Corsica appeared as an early offshoot and was strongly supported as sister to all remaining continental samples (Fig. 3). The interior topology of the latter continental clade was poorly resolved except for a terminal clade of European *C. c. caeruleus* and the island lineages from Crete, *C. c. calamensis* and from the Bristish Isles, *C. c. obscurus* (Fig. 3). The intraspecific topology of *C. cyanus* was poorly resolved in all reconstructions.

3.3. Molecular dating and ancestral range reconstructions

Among five independent dating approaches with BEAST, oldest split ages within *Cyanistes* were inferred from the multilocus data set with two outgroup calibration nodes and partitioning of the data set yielded slightly older estimates and considerably wider 95% HPD intervals (Table 3, calibrations 3, 4; Fig. 3). Age estimates inferred from cytochrome-*b* data alone, were slightly older when a fixed substitution rate was applied to the data set compared to younger estimates when fixed node ages were applied to four outgroup nodes (Table 3, calibrations 1, 2; Fig. A1). Remarkably, younger split ages were inferred from the mulitlocus data set when fixed node ages were assigned to three further *Cyanistes* ingroup nodes (equally young split ages than those inferred from cyt*b* data alone; Table 3, calibration 5).

The origin of all *Cyanistes* tits was dated to the Miocene–Pliocene boundary (4.9–6.5 Ma; calibrations 1, 3, 4), but was slightly younger according to fixed node ages (mid-Pliocene, 3.1–3.3 Ma; Table 3, calibrations 2, 5; Figs. 3 and A1). Oldest split ages for the eastern and western Palearctic sister species pair (*C. caeruleus* and *C. cyanus*) were dated to the Pliocene (2.4–4.7 Ma; Table 3, calibrations 3, 4), youngest estimates dated this split to the early Pleistocene (1.5–2.0 Ma; Table 3, calibrations 1, 2, 5; Figs. 3 and A1). Age estimates for the ancestor of the extant *C. teneriffae* also varied slightly between the different calibrations with oldest estimates at the beginning of the Pliocene (4.2–5.6 Ma; Table 3, calibrations 3, 4) compared to younger estimates at the late Pliocene (2.1–3.4 Ma; Table 3, calibrations 1, 2, 5; Figs. 3 and A1).

Likelihood and parsimony reconstructions of blue and azure tit ancestral ranges suggested partly conflicting scenarios, as shown in Fig. 4. ML reconstructions inferred a Canarian ancestral range for *Cyanistes* tits in 76.4% of all trees (Fig. 4, A1. excluding outgroups; vs. 23.4% with equivocal state; nearly 100% Canarian ancestral range when outgroups were included). An unequivocal Palearctic range was inferred for the two Eurasian species (and for their common ancestor; 81.2% eastern Palearctic origin when outgroups were excluded and the two Palearctic areas were treated as separate categories), although, with a notable percentage of equivocal states for the ancestor of *C. caeruleus* (30% equivocal vs. 44% western Palearctic origin; Fig. 4). Afrocanarian blue tits were unambiguously assigned to a Canary Island origin, as was the ancestor of the core group regardless of outgroup treatment (Fig. 4, nodes B1 and C1). At both nodes the Canarian ancestral state also had the highest average likelihood frequencies. North Africa was inferred

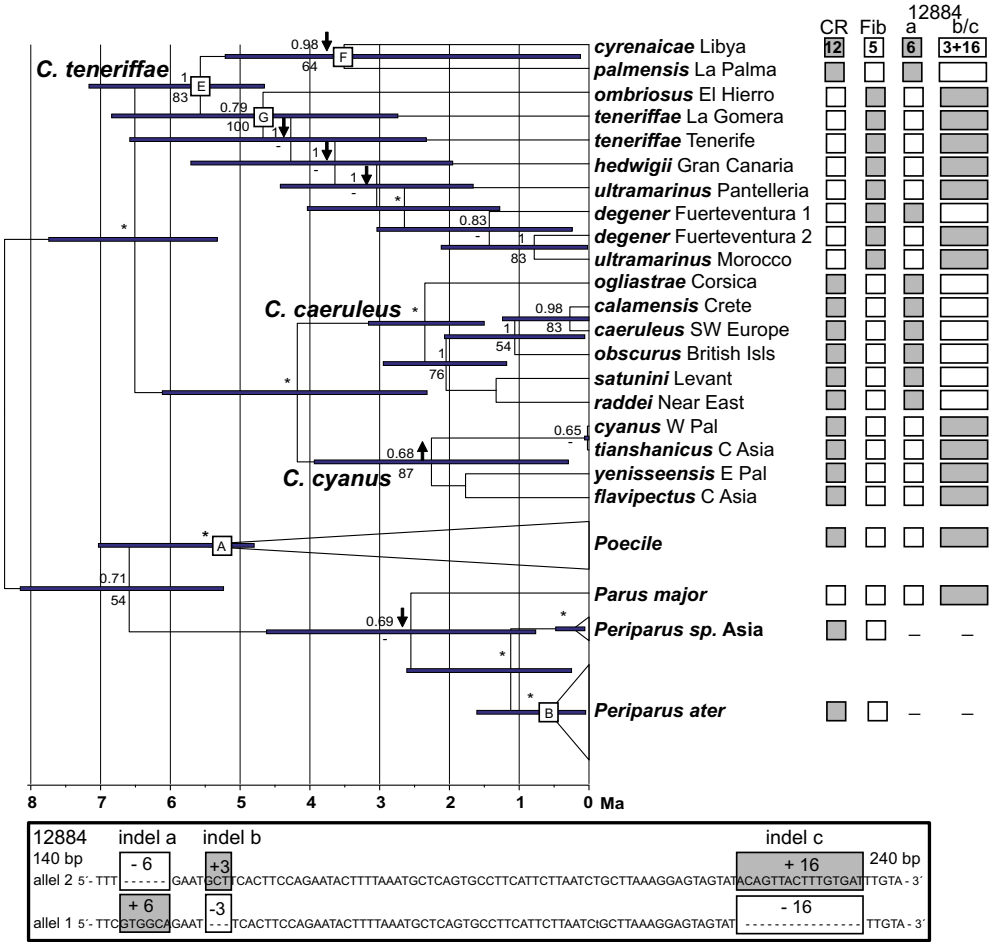

Fig. 3. Dated Bayesian phylogeny for *Cyanistes* blue and azure tits and related tit outgroups based on 2874 base pairs of concatenated sequence data (partitioned by gene and codon position). BEAST, 30,000,000 generations, burn-in = 9000, Yule prior, mean heights, posterior probabilities indicated above nodes, thorough bootstrap support from raxML analysis below nodes (– = node not present in ML analysis), asterisk denotes full node support from Bayesian analysis and bootstrap support >95% from raxML analysis; strong decrease/increase of node support from an independent BEAST analysis with an unpartitioned data set (using the same two calibration nodes A and B) indicated by downward/upward arrows. Variation of four indels shown at the respective clades (CR = control region, Fib = fibrinogen intron 7, 12,884a, b/c = three introns of the anonymous nuclear marker 12,884), same number of base pairs across all taxa as indicated in first line of boxes; gray boxes = nucleotides, blank boxes = gaps (as illustrated for a fragment of nuclear marker 12,884 below). The tree was calibrated with two fixed node ages applied to outgroup nodes A (trans-Beringia interchange) and B (Messinian Crisis); in an independent run three further ingroup nodes (E–G) were used for calibration along with the two outgroup nodes (cf. Table 3).

Table 3
Age estimates of lineage splits (in Ma) within *Cyanistes* blue and azure tits as inferred from five independent runs with BEAST 1.4.8, means and [95% HPD intervals]; Markov chain length 30,000,000; partition by codon for both runs with cytochrome-*b* data; full Paridae cytochrome-*b* data set, fixed rate ($r = 0.0105$); full Paridae cytochrome-*b* data set, four fixed node ages (A, C: trans-Beringia faunal interchange [two Nearctic/Palearctic splits in *Poecile* and *Lophohporus*/*Baleolophus* respectively], B: Messinian Crisis, basal split of Mediterranean coal tits, [*Periparus ater*]; D: age of Taiwan [split of *Parus holsti*]); reduced multigene data set (unpartitioned and partitioned) with two fixed node ages applied to outgroup clades (nodes A, *Poecile*, and B, *Periparus ater*) and a further independent run with five fixed node ages applied (nodes A, B + three internal *Cyanistes* nodes: volcanic ages of La Palma, El Hierro applied to the respective splits and the beginning of the Messinian Crisis applied to the basal split in *Cyanistes*; compare Figs. 3 and A1).

Calibration Split	(1) cyt*b* $r = 0.0105$ by codon	(2) cyt*b* 4 nodes by codon 4 nodes (out)	(3) 5 genes no partition 2 nodes (out)	(4) 5 genes by gene and codon 2 nodes (out)	(5) 5 genes no partition 5 nodes (out/in)
Cyanistes	4.9 [2.0–6.1]	3.1 [2.3–4.8]	5.9 [2.7–9.8]	6.5 [5.3–7.7]	3.1 [1.8–4.5]
C. teneriffae	3.4 [3.2–6.5]	2.1 [1.5–3.4]	4.2 [1.9–7.1]	5.6 [4.7–7.2]	2.1 [1.3–3.1]
Crown group	1.3 [0.8–2.0]	1.0 [0.5–1.4]	2.3 [1.0–3.9]	4.7 [2.7–6.8]	1.0 [0.8–1.1]
C. caeruleus/*C. cyanus*	2.0 [1.1–2.9]	1.5 [0.8–2.3]	2.9 [1.2–4.9]	4.2 [2.3–6.1]	1.6 [0.8–2.4]
C. caeruleus (*ogliastrae* Corsica vs. others)	0.9 [0.5–1.3]	0.8 [0.3–1.0]	1.4 [0.6–2.5]	2.4 [1.5–3.2]	0.8 [0.4–1.3]

as a possible ancestral range at only one node of the entire *C. teneriffae* clade, and this in only a small percentage of trees when outgroups were excluded (33% vs. 47.7% Canarian ancestral range for the terminal Afrocanarian clade; Fig. 4).

Parsimony reconstruction yielded a different scenario: A western Palearctic ancestral range was inferred for the entire *Cyanistes* tit group (Fig. 4, node A2), in which case European blue tits originated from a western Palearctic range, while azure tits originated from an eastern Palearctic ancestral range (the ancestor of both species was inferred to have had a western range). The origin of Afrocanarian tits was highly equivocal, although in 18.2% of all trees North Africa was identified as the uniquely best state at node B2 (Fig. 4). In the remaining 81.8% of trees the character state was equivocal at node B2, i.e. North Africa and the Canary Islands were

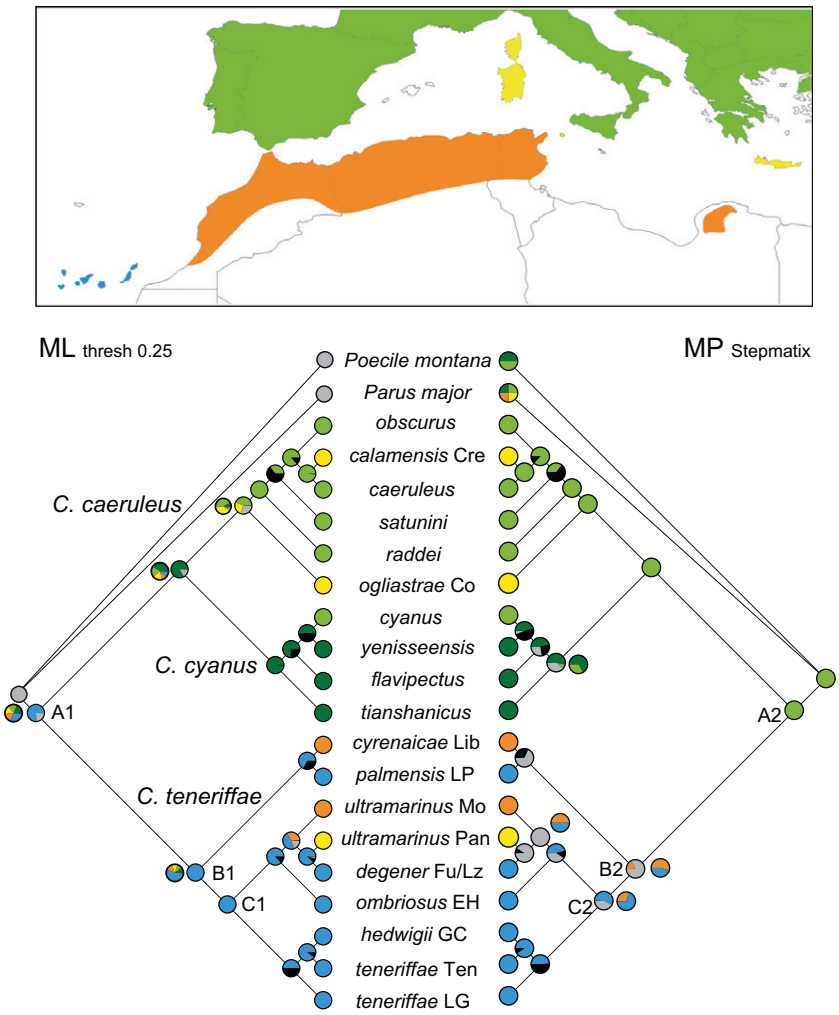

Fig. 4. Ancestral area reconstruction for *Cyanistes* blue and azure tits with Mesquite, option "trace character over trees" using the Bayesian tree as a phylogenetic backbone and characters traced over 21,000 trees from MrBayes run (after excluding a burn-in of the first 9000 trees). Left = ML reconstruction (outgroups excluded) with likelihood threshold of 0.25, pie charts accounting for average state frequencies across trees given at the left of some nodes (root, A1–C1 and basal Palearctic nodes). Right = MP reconstruction with stepmatrix model applied, pie charts counting "all trees with state" given at the right of some nodes (see above). Geographic areas indicated by color codes according to the map: eastern Palearctic = dark green, extralimital, not shown on map. Black = node absent, gray = equivocal character state (e.g. two equally parsimonious states). Area abbreviations: Cre = Crete, Co = Corsica, EH = El Hierro, Fu/Lz = Fuerteventura/Lanzarote, GC = Gran Canaria, LG = La Gomera, Lib = Libya, LP = La Palma, Mo = Morocco, Pan = Pantelleria, Ten = Tenerife.

both within the optimal set and equally parsimonious (calculate option: "count all trees with state"; Fig. 4, B2). However, it is noteworthy that the Canary Islands were never counted as the uniquely best state at node B2. The ancestor of the core group was inferred to have a Canary Island range in 57.5% of the trees (vs. 42.2% equivocal state) and the island range was always the uniquely best state compared to North Africa (only 29.9% of all trees having a state; Fig. 4, C2).

4. Discussion

4.1. Historical biogeography – colonization of the Canary Islands

Although phylogenetic relationships among *Cyanistes* blue and azure tits have been previously studied in detail based on a number of molecular markers (Dietzen et al., 2008; Illera et al., 2011; Kvist et al., 2005; Salzburger et al., 2002) inclusion of Libyan *C. t. cyrenaicae* into the molecular data set has revealed an unexpectedly complex and puzzling phylogeographic scenario. There is no such strong genetic differentiation among allopatric North African populations in coal tits (*Periparus ater*; Tietze et al., 2011) and North African great tits (*Parus major excelsus*) are not genetically distinct from their western European conspecifics (Päckert et al., 2005). In contrast, intraspecific genetic differentiation between eastern and western North African populations has been detected in two lark species (García et al., 2008; Guillaumet et al., 2006).

The phylogenetically basal position of the two peripheral *C. teneriffae* populations suggests that relic lineages from the initial early Pliocene Afrocanarian faunal interchange have survived in these regions. Continuous forest breeding ranges must have become fragmented as climate cooling and desertification progressed in North Africa approaching the Pleistocene boundary (Axelrod and Raven, 1978; Sanmartín et al., 2010) and a northeastern African forest refuge in the Libyan Cyrenaica might have harbored the ancestors of extant *C. t. cyrenaicae*. Palaeofaunal reconstructions by Jost et al. (2009) supported such a late Pliocene eastern refuge over wide regions with dense forest cover in northeastern Africa, rather than in northwestern Africa. Moreover, a Bayesian phylogeographic analysis predicted the greatest carrying capacity on either the western Canary Islands or on the North African mainland, depending on the model parameters applied (Sanmartín et al., 2008). Thus, long-term refuges can be expected in these peripheral populations because a species' survival depends, amongst other things, on a refugium's carrying capacity (Steward et al., 2010).

Previous suggestions regarding the geographic origin of Afrocanarian blue tits, based on phylogeographic analysis, were either highly equivocal (Tietze and Borthakur, 2012) or open to multiple interpretations (Illera et al., 2011), as were our own. We believe that likelihood reconstructions have less explanatory power because: (1) in contrast to the stepmatrix model, state changes are equally probable in the Mk1 model; and (2) character state information for outgroups cannot be appropriately categorized in ML reconstructions. In particular, uncertainty regarding outgroup relationships may lead to uncertainty regarding ancestral states (Maddison et al., 1984). Therefore, we favor an ancestral range scenario based on parsimony reconstructions in the following proposition: An initial colonization from the European continent (ancestor of all *Cyanistes*) to North Africa was followed by further colonization of the Canary Islands (with highly equivocal results for the ancestor of *C. teneriffae*). Regardless of the reconstruction method applied, the continental populations from Morocco and Algeria certainly originated from a back-colonization event from the Canarian Archipelago to North Africa.

Multiple faunal interchanges between the Canary Islands and the North African and/or European continent have been suggested for other passerines such as finches (*Fringilla;* Marshall and Baker, 1999; Rando et al., 2010), robins (*Erithacus*; Dietzen et al., 2003) and stonechats (*Saxicola*; Illera et al., 2008). In particular, chaffinches and robins have been more or less explicitly treated as examples of 'downstream colonization' (moving from continents to islands; e.g. chaffinch sink populations on the Canary Islands; Samarassin-Dissanayake, 2010). The blue tit island population of Corsica was similarly been considered a sink population based on the fact that their breeding phenology is ecologically adapted to relatively low quality habitats (Blondel et al., 1992). However, there is recent evidence that significant genetic differentiation among local Corsican blue tit populations was best explained by adaptation to different habitat types (Porlier et al., 2012).

In contrast, 'upstream colonization' (from islands to adjacent continents) seems to be much rarer (review in Bellemain and Ricklefs, 2008) but has been documented in some Pacific and Atlantic bird groups (Filardi and Moyle, 2005; Bellemain et al., 2008; Jønsson et al., 2011), in some plants (Carine et al., 2004; Hutsemékers et al., 2011) and in Macronesian invertebrates (Greve et al., 2010).

4.2. Inter-island colonization pathways and differentiation

In a previous phylogeographic study Illera et al. (2011) discussed several explanations for the genetic admixture among the three *Cyanistes* species, including ancient polymorphism and male-mediated gene flow among European and Canary Island populations. Mapping the four indels onto the multigene tree, however, suggested that different alleles were fixed in the peripheral populations and in the core group, while some populations in the latter group have retained their polymorphism to the present day. The respective nuclear haplotypes therefore probably represent traces of early continental–island faunal interchanges and incomplete lineage sorting in the Fuerteventura and Moroccan populations. In birds, incomplete lineage sorting has been typically reported in a number of Palearctic sister species believed to have originated from relatively young Pleistocene speciation events (Marthinsen et al., 2008; Maley and Winker, 2010; Päckert et al., 2012a). Alternatively, intraspecific genetic admixture between allopatric *C. teneriffae* populations and interspecific admixture between Afrocanarian blue tits and the other two Eurasian species (nuclear markers) might provide evidence of introgression due to extant or even past hybridization in Pleistocene refuges (Liebers et al., 2004; Babik et al., 2005; Sternkopf et al., 2010). Significant migration rates among *C. caeruleus* and *C. teneriffae* inferred from coalescent analyses under the isolation with migration model of largely the same data set (excluding *C. t. cyrenaicae*; Illera et al., 2011) suggested a scenario of gene flow after their divergence, while microsatellite analysis suggested rather limited contemporary gene flow among Canary Island populations and those on the adjacent continents (even among the eastern islands and North Africa; Illera et al., 2011). In fact, discordances among phylogenetic trees inferred from mtDNA and nuclear markers might well be caused by a combination of both introgression and incomplete lineage sorting (McGuire et al., 2007).

Furthermore, mitochondrial introgression provides a plausible explanation for the strong discrepancies among nuclear and mitochondrial trees/networks with respect to the La Gomera population. Considering the very close proximity to neighboring Tenerife, mitochondrial introgression through past hybridization processes might be expected among these islands.

In summary, our results suggest that the complex phylogeographic pattern in *Cyanistes* tits results from a number of processes including incomplete lineage sorting, drift, mitochondrial introgression, and possibly selection as well. Furthermore, complex colonization patterns might be obscured by vicariance events due to multiple downstream colonization, inter-island dispersal and within-island speciation (Juan et al., 2000; Carranza et al., 2002; Bellemain et al., 2008; Sanmartín et al., 2008), as well as local extinction events on islands such as those demonstrated for some Canarian species groups of lizards (Maca-Meyer et al., 2003) and birds (Ramírez et al., 2010; Rando et al., 2010; Illera et al., 2012). Despite all this, our dated *Cyanistes* phylogeny revealed a clear colonization scenario involving an initial downstream colonization event (with La Palma as a relic population) and a terminal back-colonization to North Africa from eastern Canary Islands (upstream colonization). Colonization pathways within the Canarian Archipelago yet remain obscure because an eastward stepping-stone colonization from El Hierro towards Lanzarote and Fuerteventura was (strongly) supported only by the partitioned multilocus data set calibrated with BEAST. Island stepping-stone colonization has also been confirmed for some other Canary Islands species groups of invertebrates (Hochkirch and Görzig, 2008; Suárez et al., 2009) and *Gallotia* lizards (Cox et al., 2010).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.02.010.

References

Axelrod, D.I., Raven, P.H., 1978. Late Cretaceous and Tertiary vegetation history of Africa. In: Werger, M.J.A. (Ed.), Biogeography and Ecology of Southern Africa. Junk, The Hague, The Netherlands, pp. 77–130.

Babik, W., Branicki, W., Crnobrnja-Isailović, J., Cogălniceanu, D., Sas, I., Olgun, K., Poykariv, N.A., Garcia-París, M., Arntzen, J.W., 2005. Phylogeography of two

European newt species – discordance between mtDNA and morphology. Mol. Ecol. 14, 2475–2491.
Backström, N., Fagerberg, S., Ellegren, H., 2008. Genomics of natural bird populations: a gene-based set of reference markers evenly spread across the avian genome. Mol. Ecol. 17, 964–980.
Barrientos, R., Kvist, L., Barbosa, A., Valera, F., López-Iborra, G.M., Moreno, E., 2009. Colonization patterns and genetic structure of peripheral populations of the trumpeter finch (*Bucanetes githagineus*) from Northwest Africa, the Canary Islands and the Iberian Peninsula. J. Biogeogr. 36, 210–219.
Bellemain, E., Ricklefs, R.E., 2008. Are islands the end of the colonization road? Trends Ecol. Evol. 23, 461–468.
Bellemain, E., Bermingham, E., Ricklefs, R.E., 2008. The dynamic evolutionary history of the bananaquit (*Coereba flaveola*) in the Caribbean revealed by a multigene analysis. BMC Evol. Biol. 8, 240.
Blondel, J., Perret, P., Maistre, M., Dias, P.C., 1992. Do harlequin environments function as a source sink for blue tits (*Parus caeruleus* L.)? Landscape Ecol. 6, 213–219.
Broggi, J., Hohtola, E., Orell, M., Nilsson, J.A., 2005. Local adaptation to winter conditions in a passerine spreading north: a common garden approach. Evolution 59, 1600–1603.
Burg, T.M., Gaston, A.J., Winker, K., Friesen, V.L., 2006. Effects of Pleistocene glaciations on populations structure of North American chestnut-backed chickadees. Mol. Ecol. 15, 2409–2419.
Carine, M.A., Russell, S.J., Santos-Guerra, A., Francisco-Ortega, J., 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Colvolvulus* (Convolvulaceae). Am. J. Bot. 91, 1070–1085.
Carranza, S., Arnold, E.N., Mateo, J.A., Geniez, P., 2002. Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol. 23, 244–256.
Cassel-Lundhagen, A., Tammaru, T., Windig, J.J., Ryrholm, N., Nylin, S., 2009. Are peripheral populations special? Congruent patterns in two butterfly species. Ecography 32, 591–600.
Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.
Cox, S.C., Carranza, S., Brown, R.P., 2010. Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Mol. Phylogenet. Evol. 56, 747–757.
Cramp, S., Perrins, C.M., 1993. Handbook of the Birds of Europe the Middle East and North Africa. Vol. VII, Flycatchers to Shrikes. Oxford University Press, Oxford, New York, pp. 225–248.
Del Hoyo, J., Elliott, A., Christie, D.A., 2007. Handbook of the Birds of the World (HBW). Vol. 12, Picathartes to Tits and Chickadees. Lynx Edicions, Barcelona.
Dickinson, E., 2003. The Howard and Moore Complete Checklist of the Birds of the World. Princeton University Press, Princeton and Oxford.
Dietzen, C., Witt, H.-H., Wink, M., 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 Sci. 2 and 3, 115–131.
Dietzen, C., García-del-Rey, E., Castro, G.D., Wink, M., 2008. Phylogeography of the blue tit (*Parus teneriffae*-group) on the Canary Islands based on mitochondrial DNA sequence data and morphometrics. J. Ornithol. 149, 1–12.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Emerson, B.C., 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding patterns and processes. Mol. Ecol. 11, 951–966.
Emerson, B.C., Oromí, P., Hewitt, G.M., 2000. Colonization and diversification of the species *Brachyderes rugatus* (Coleoptera) on the Canary Islands: evidence from mitochondrial DNA COII gene sequences. Evolution 54, 911–923.
Filardi, C.E., Moyle, R.G., 2005. Single origin of a pan-Pacific bird group and upstream colonization of Australasia. Nature 438, 216–219.
García, J.T., Suárez, F., Garza, V., Calero-Riestra, M., Hernández, J., Pérez-Tris, J., 2008. Genotypic and phenotypic variation among geographically isolated populations of the globally threatened Duponťs lark *Chersophilus duponti*. Mol. Phylogenet. Evol. 46, 237–251.
Garland, T., Adolph, S.C., 1991. Physiological differentiation of vertebrate populations. Ann. Rev. Ecol. Syst. 22, 193–228.
Gill, F.B., Slikas, B., Sheldon, F.H., 2005. Phylogeny of titmice (Paridae): II. Species relationships based on sequences of the mitochondrial cytochrome-*b* gene. Auk 122, 121–143.
Greve, C., Hutterer, R., Groh, K., Haase, M., Misof, B., 2010. Evolutionary diversification of the genus *Theba* (Gastropoda: Helicidae) in space and time: a land snail conquering islands and continents. Mol. Phylogenet. Evol. 57, 572–584.
Guillaumet, A., Pons, J.M., Godelle, B., Crochet, P.A., 2006. History of the crested lark in the Mediterranean region as revealed by mtDNA sequences and morphology. Mol. Phylogenet. Evol. 39, 645–656.
Hardie, D.C., Hutchings, J.A., 2010. Evolutionary ecology at the extremes of speciеś ranges. Environ. Rev. 18, 1–20.
Hochkirch, A., Görzig, Y., 2008. Colonization and speciation on volcanic islands: phylogeography of the flightless grasshopper genus *Arminda* (Orthoptera, Acrididae) on the Canary Islands. Syst. Entomol. 34, 188–197.
Hutsemékers, V., Szövényi, P., Shaw, A.J., González, J.-M., Muños, J., Vanderpoorten, A., 2011. Oceanic islands are not sinks of biodiversity in spore-producing plants. Proc. Natl. Acad. Sci. USA. 108, 18989–18994.
Illera, J.C., Emerson, B.C., Richardson, D.S., 2007. Population history of Berthelot's pipit: colonisation, gene flow and morphological divergence in Macaronesia. Mol. Ecol. 16, 4599–4612.
Illera, J.C., Richardson, D.S., Helm, B., Atienza, J.C., Emerson, B.C., 2008. Phylogenetic relationships, biogeography and speciation in the avian genus *Saxicola*. Mol. Phylogenet. Evol. 48, 1145–1154.
Illera, J.C., Koivula, K., Broggi, J., Päckert, M., Martens, J., Kvist, L., 2011. A multi-gene approach reveals a complex evolutionary history in the *Cyanistes* species group. Mol. Ecol. 20, 4123–4139.
Illera, J.C., Rando, J.C., Richardson, D.S., Emerson, B.C., 2012. Age, origins and extinctions of the avifauna of Macaronesia: a synthesis of phylogenetic and fossil information. Quartern. Sci. Rev. 50, 14–22.
Johnson, K.P., 2001. Taxon sampling and the phylogenetic position of Passeriformes: evidence from 916 avian cytochrome-*b* sequences. Syst. Biol. 50, 128–136.
Jønsson, K.A., Fabre, P.-H., Ricklefs, R.E., Fjeldså, J., 2011. Major global radiation of corvoid birds originated in the proto-Papuan archipelago. Proc. Natl. Acad. Sci. USA 108, 2328–2333.
Jost, A., Fauquette, S., Kageyama, M., Krinner, G., Ramstein, G., Suc, J.-P., Violette, S., 2009. High resolution climate and vegetation simulations of the Late Pliocene, a model-data comparison over western Europe and the Mediterranean region. Clim. Past 5, 585–606.
Juan, C., Emerson, B.C., Oromí, P., Hewitt, G.M., 2000. Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. Trends Ecol. Evol. 15, 104–109.
Kvist, L., Ruokonen, M., Lumme, J., Orell, M., 1999. Different population structures in northern and southern populations of the European blue tit (*Parus caeruleus*). J. Evol. Biol. 12, 798–805.
Kvist, L., Broggi, J., Illera, J.C., Koivula, K., 2005. Colonisation and diversification of the blue tits (*Parus caeruleus teneriffae*-group) in the Canary Islands. Mol. Phylogenet. Evol. 34, 501–511.
Lehtonen, P.K., Laaksonen, T., Artemyev, A.V., Belskii, E., Both, C., Bureš, S., Bushuev, A.V., Krams, I., Moreno, J., Mägi, M., Nord, A., Potti, J., Ravussin, P.-A., Sirkiä, P.M., Sætre, G.-P., 2009. Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). Mol. Ecol. 18, 4463–4476.
Liebers, P.K., de Knijf, P., Helbig, A.J., 2004. The Herring Gull complex is not a ring species. Proc. R. Soc. Lond. B271, 893–901.
Maca-Meyer, N., Carranza, S., Rando, J.C., Arnold, E.N., Cabrera, V.M., 2003. Status and relationships of the extinct giant Canary Island lizard *Gallotia goliath* (Reptilia: Lacertidae), assessed using ancient mtDNA from its mummified remains. Biol. J. Linn. Soc. 80, 659–670.
Maddison, W.P., Maddison, D.R., 2008. Mesquite: a Modular System for Evolutionary Analysis, Version 2.5. <http://mesquiteproject.org>.
Maddison, W.O., Donoghue, M.J., Maddison, D.R., 1984. Outgroup analysis and parsimony. Syst. Zool. 33, 83–103.
Maley, J., Winker, K., 2010. Diversification at high latitudes: speciation of buntings in the genus *Plectrophenax* inferred from mitochondrial and nuclear markers. Mol. Ecol. 19, 785–795.
Marshall, H.D., Baker, A.J., 1999. Colonization history of Atlantic island common chaffinches (*Fringilla coelebs*) revealed by mitochondrial DNA. Mol. Phylogenet. Evol. 11, 201–212.
Marthinsen, G., Wennerberg, L., Lijfjeld, J.T., 2008. Low support for separate species within the redpoll complex (*Carduelis flammea-hornemanni-cabaret*) from analyses of mtDNA and microsatellite markers. Mol. Phylogenet. Evol. 47, 1005–1007.
McGuire, J.A., Linkem, C.W., Koo, M.S., Hutchison, D.W., Lappin, K., Orange, D.I., Lemos-Espinal, L., Riddle, B.R., Jaeger, J.R., 2007. Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. Evolution 61, 2879–2897.
Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
Omland, K.E., Lanyon, S.M., Fritz, S., 1999. A molecular phylogeny of the New World orioles (*Icterus*): the importance of dense taxon sampling. Mol. Phylogenet. Evol. 12, 224–239.
Päckert, M., Martens, J., Nazarenko, A.A., Valchuk, O., Petri, B., Eck, S., Veith, M., 2005. The great tit, *Parus major*, a misclassified ring species. Biol. J. Linn. Soc. 86, 153–174.
Päckert, M., Dietzen, C., Martens, J., Wink, M., Kvist, L., 2006. Radiation of Atlantic goldcrests *Regulus regulus* spp.: evidence of a new taxon from the Canary Islands. J. Avian Biol. 37, 364–380.
Päckert, M., Martens, J., Wink, M., Feigl, A., Tietze, D.T., 2012a. Molecular Phylogeny of Old World swifts (Aves: Apodiformes, Apodidae, *Apus* and *Tachymarptis*) based on mitochondrial and nuclear markers. Mol. Phylogenet. Evol. 63, 606–616.
Päckert, M., Martens, J., Sun, Y.-H., Severinghaus, L.L., Nazarenko, A.A., Ting, J., Töpfer, T., Tietze, D.T., 2012b. Horizontal and elevational patterns of Himalayan and Southeast Asian forest passerines (Aves: Passeriformes). J. Biogeogr. 39, 556–573.
Pagel, M., 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48, 612–622.
Pandey, M., Rajora, O.P., 2012a. Genetic diversity and differentiation of core vs. peripheral populations of eastern white cedar, *Thuja occidentalis* (Cupressaceae). Am. J. Bot. 99, 690–699.
Pandey, M., Rajora, O.P., 2012b. Higher fine scale genetic structure in peripheral than in core populations of a long-lived and mixed-mating conifer – eastern white cedar (*Thuja occidentalis* L.). BMC Evol. Biol. 12, 14.

Piñeiro, R., Widmer, A., Fuertes Aguilar, J., Nieto Feliner, G., 2011. Introgression in peripheral populations and colonization shape the genetic structure of the coastal shrub *Armeria pungens*. Heredity 106, 228–240.

Porlier, M., Garant, D., Perret, P., Charmantier, A., 2012. Habitat-linked population genetic differentiation in the blue tit *Cyanistes caeruleus*. J. Hered. 103, 781–791.

Quaisser, C., Eck, S., 2003. *Parus montanus* Conrad, 1827. Weidenmeise. In: Eck, S., Martens, J. Sun, Y.-H. (Eds.), Atlas der Verbreitung Paläarktischer Vögel, 20. Lieferung. Erwin-Stresemann-Gesellschaft für paläarktische Avifaunistik e.v., Berlin.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.

Ramírez, O., Illera, J.C., Rando, J.C., González-Solís, J., Alcover, J.A., Laluéza-Fox, C., 2010. Ancient DNA of the extinct lava shearwater (*Puffinus olsoni*) from the Canary Islands reveals incipient differentiation within the *P. puffinus* complex. PLoS One 5 (12), e16072.

Rando, J.C., Alcover, J.A., Illera, J.C., 2010. Disentangling ancient interactions: a new extinct passerine provides insights on character displacement among extinct and extant island finches. PLoS One 5, e12956.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Salzburger, W., Martens, J., Sturmbauer, C., 2002. Paraphyly of the blue tit (*Parus caeruleus*) suggested from cytochrome-*b* sequences. Mol. Phylogenet. Evol. 24, 19–25.

Samarassin-Dissanayake, P., 2010. Population Differentiation, Historical Demography and Evolutionary Relationships among Widespread Common Chaffinch Populations (*Fringilla coelebs* ssp.). PhD Thesis, Department of Ecology and Evolutionary Biology, University of Toronto.

Sanmartín, I., van der Mark, P., Ronquist, F., 2008. Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. J. Biogeogr. 35, 428–449.

Sanmartín, I., Anderson, C.L., Alarcon, M., Ronquist, F., Aldasoro, J.J., 2010. Bayesian island biogeography in a continental setting: the Rand Flora case. Biol. Lett. 6, 703–707.

Schluter, D., Price, T., Mooers, A.O., Ludwig, D., 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51, 1699–1711.

Schwartz, M.K., Mills, L.S., Ortega, Y., Ruggiero, L.F., Allendorf, F.W., 2003. Landscape location affects genetic variation of Canada lynx (*Lynx canadensis*). Mol. Ecol. 12, 1807–1816.

Silvestro, D., Michalak, I., 2010. raxmlGUI: a Graphical Front-end for RAxML. <http://sourceforge.net/projects/raxmlgui/>.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Sternkopf, V., Liebers-Helbig, D., Ritz, M.S., Zhang, J., Helbig, A.J., de Knijf, P., 2010. Introgressive hybridization and the evolutionary history of the herring gull complex revealed by mitochondrial and nuclear DNA. BMC Evol. Biol. 10, 348.

Steward, J.R., Lister, A.M., Barnes, I., Dalén, L., 2010. Refugia revisited: individualistic responses of species in space and time. Proc. R. Soc. Lond. B 277, 661–671.

Suárez, N.M., Betancor, E., Pestano, J., 2009. Intraspecific evolution of Canarian *Euchloe* (Lepidoptera: Pieridae) butterflies, based on mtDNA sequences. Mol. Phylogenet. Evol. 51, 601–605.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Tietze, D.T., Borthakur, U., 2012. Phylogeography of tits (Aves: Paridae, Remizidae). Organ. Divers. Evol. 12, 433–444.

Tietze, D.T., Martens, J., Sun, Y.H., Severinghaus, L.L., Päckert, M., 2011. Song evolution in the coal tit. J. Avian Biol. 42, 1–23.

Tomozawa, M., Suzuki, H., 2008. A trend of central versus peripheral structuring in mitochondrial and nuclear gene sequences of the Japanese wood mouse *Apodemus speciosus*. Zool. Sci. 25, 273–285.

Warren, B.H., Bermingham, E., Bourgeois, Y., Estep, L.K., Prŷs-Jones, R.P., Strasberg, D., Thébaud, C., 2012. Hybridization and barriers to gene flow in an island bird radiation. Evolution 66, 1490–1505.

Weir, J.T., Schluter, D., 2008. Calibrating the avian molecular clock. Mol. Ecol. 17, 2321–2328.