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Quaternary history of an endemic passerine bird on Corsica Island: Glacial refugium and impact of recent forest regression



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ABSTRACT

Molecular studies support the hypothesis that Corsica Island was a glacial refugium for a number of forest birds during the Pleistocene. We focused on the Corsican nuthatch (*Sitta whiteheadi*), an endemic passerine strongly associated with the laricio pine (*Pinus nigra laricio*). The range of laricio pine has been impacted by the Pleistocene glacial periods and forest has been recently fragmented by cutting and fires. Using both molecular (mitochondrial and nuclear) and morphological characters, we assessed the variation within the nuthatch population. Our results are consistent with the hypothesis that the Corsican nuthatch endured through the late Pleistocene and Holocene climatic variations, and sustained the subsequent cycles of forests reduction/expansion. The results also suggest that the recent anthropization of the landscape resulted in the isolation of a cluster of populations in the northern part of the island. The fragmentation of the habitat of the nuthatch may impede the future of the bird by creating isolated population units between which the gene flow is reduced.

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Introduction

Successive glacial and interglacial events have impacted the geographical distribution of many plant or animal taxa in Europe (Prentice et al., 2000; Magri et al., 2006). These repeated phases of range contraction and expansion have favored genetic differentiation among populations of Palearctic fauna and flora (Hewitt, 2004; Schmitt, 2007). In the Mediterranean, three major refugia have been described (in the Iberian and Italian peninsulas, and in the Balkans), from where the populations recolonized northern areas during warmer periods (Taberlet et al., 1998; Brito, 2005). Molecular studies also support the hypothesis that some of the largest Mediterranean islands, i.e., Sardinia and Corsica, have played the role of refugium for birds during these climatic events (Kvist et al., 2004; Pons et al., 2015). The endemic Corsican nuthatch (*Sitta whiteheadi*) is presumably an old element of the island's avifauna. Its divergence with its sister-species, the Chinese nuthatch (*Sitta villosa*, from the far east Palearctic, in China, North and South Korea), date to 1 Ma (Pasquet et al., 2014). Such disjunct and extremely distant ranges between Europe and Asia are rare among closely related species, one of the most famous example being the Azure-winged Magpies complex (*Cyanopica cyanus* from Asia and *C. cooki* from Spain) with an estimate of divergence time between

the late Pliocene and the early Pleistocene (Fok et al., 2002; Kryukov et al., 2004). The Corsican nuthatch belongs to the group of nuthatches that are closely associated with pine forests and depend almost exclusively on cone seeds, especially during winter (Pasquet et al., 2014). It is tightly associated with the laricio pine (*Pinus nigra laricio*), a subspecies distributed in Corsica, South Italy and Sicily (Farjon and Filer, 2013), removing seeds from cones when they are mature and hiding them on branches and under the bark of trunks, to retrieve them during cold days when cones are closed (Thibault et al., 2006). Territories are occupied year-round by the couple, up to 1800 m in altitude, i.e., the highest limit of the forest. This dependence on seeds implies the presence of a sufficient amount of mature pines to provide a sufficient amount of resources throughout the year. Adults and non-breeders are strictly sedentary, rarely recorded outside the laricio pine range (Thibault et al., 2006). The Corsican nuthatch is considered as a "vulnerable" species (BirdLife International, 2013), with a current number of territories estimated at 1557–2201, distributed on only 18.5 km² (Thibault et al., 2011). The low number of its population has been clearly linked with that of the laricio pine, the range which has been heavily fragmented by cutting and fires for cultivation and grazing (see Discussion). Today, the area where it is the dominant tree covers less 22 km² (ONF, 2006). Thus, the laricio pine and the Corsican nuthatch have had a long common history on the island, and their common decline is a reason of concern. In this paper, we used molecular and morphological characters to assess the variation in the nuthatch population that

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could be related to its Quaternary history on the island, in relation with what is known of the past and present distribution of the laricio pine.

Methods

Study area

Located in the Western Mediterranean region, Corsica Island (42°N, 9°S) encompasses an 8722 km² area, 183 km in length and 83 km in breadth. The island is notable for its mountainous landscape, with a mean altitude of 568 m, several peaks over 2000 m, and the highest summit culminating at 2706 m (Cintu Mount) (Simi, 1982). During glacial periods of the late Quaternary, sea level dropped at least 100 m around the island (Peltier, 1994). The topography of the sea bed suggests that Corsica was connected several times to Sardinia but connection to the Italian islands or coast remains debatable (Conchon, 1975; Shackleton et al., 1984).

Sampling

We sampled 220 individuals of Corsican nuthatch (hereafter “the nuthatch”) from 13 localities spanning the whole species range, which mirrors the range of the laricio pine (hereafter “the pine”) (Fig. 1). These pine forests cover today less than 2.5% of the area of Corsica (ONF, 2006). Forest stands that show present-day connectivity were treated as a single locality (example: Vizzavona and Rospa Sorba; Table 1). Birds were caught with mist-nets, using playback of recorded songs, and blood and/or feather was sampled for genetic analyses. All the individuals were released in the place of capture. Because of the strong territorial habits of nuthatches, almost all the individuals attracted and caught were males. Genomic DNA was extracted using standard protocols.

Microsatellite genotyping

We genotyped 10 microsatellite loci, eight of which originally developed for the Brown-headed nuthatch *Sitta pusilla* (SpuL5-22, SpuA6, Spu4-C6, SpuE19, Spu6-26, SpuL6-16, SpuL4-3, Spu4-E7; Haas et al., 2009) and two for other passerines (Syl14 for *Sylvia* warblers, Segelbacher et al., 2008; Pij23 for *Phylloscopus* warblers, Saito et al., 2005) (Table S1 in the supplementary material). Four of them were also used for studying the phylogeography of the Krüper’s nuthatch (*Sitta krueperi*; SpuL5-22, Syl14, Pij23, and Spu4-E7; Albayrak et al., 2012). Microsatellite fragments were amplified using a touch-down PCR as described in the original publications (Saito et al., 2005; Segelbacher et al., 2008; Haas et al., 2009). Genotyping was conducted on an ABI 3700 Genetic Analyzer (Applied Biosystem) using multiplex PCR (Qiagen). Results were visualized using Peak Scanner (Applied Biosystem).

Mitochondrial DNA sequencing

We amplified a portion of the NADH dehydrogenase subunit 2 (ND2) using the primers L5219Met 5'-CCC ATA CCC CGA AAA TGA TG-3' (Fuchs et al., 2005) and H5766s 5'-GAT GAG AAG GCY AGG ATT TTT CG-3' (Price et al., 2004). PCR amplifications were performed in 25 µl reactions with 2 µl of template and 0.4 µM final concentration for primers, using Qiagen Taq. The thermo-cycling procedure commenced with an initial denaturation of 3 minutes at 95°C, followed by 40 cycles of 40 seconds at 95°C, 40 seconds at 50°C, and 60 seconds at 72°C for elongation. PCR were purified and sequenced in both directions at contract sequencing facilities (Macrogen Europe and Eurofin) using the same primers as used in the PCR. Sequences were examined using Sequencher (Genecodes, Ann Arbor, MI, USA).

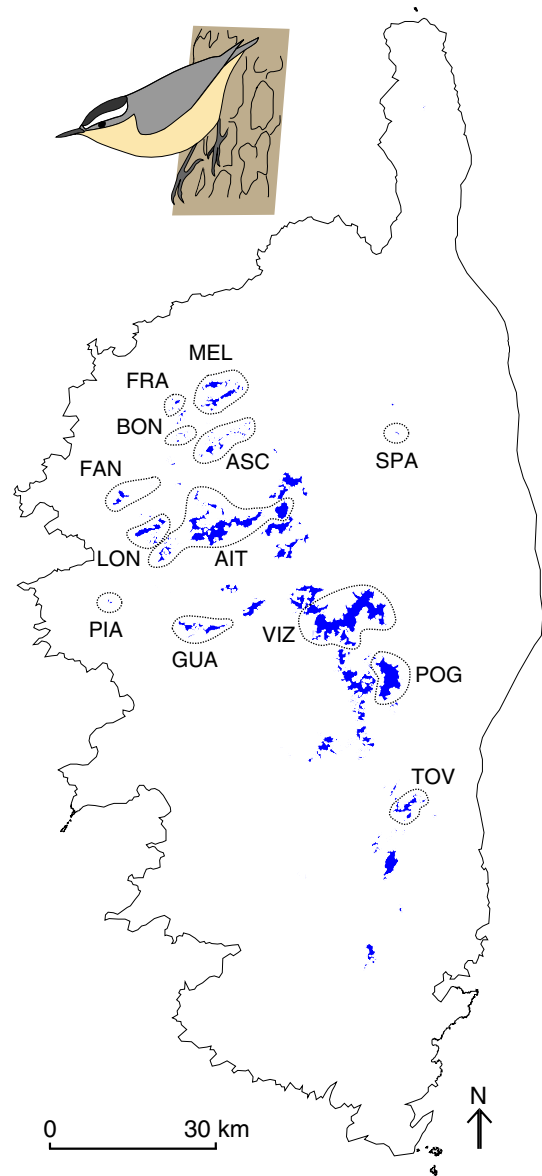


Fig. 1. Localities sampled in this study and present-day distribution of the pine (in blue, from Anon, 2003), which represents also to the distribution of the nuthatch populations.

Table 1

Localities included in this study and number of individuals sampled.

Localities	Symbols	N
Aitone + Valdoniellu + Melu	AIT	63
Lonca + Tusella	LON	8
Ascu	ASC	22
Bonifatu + Melaghia	BON	16
Fratte (Calenzana)	FRA	15
Guagnu	GUA	15
Tartagine + Melaja	MEL	20
Poggio di Nazza + Pietrapiana	POG	15
Tova	TOV	15
Vizzavona + Rospa Sorba	VIZ	27
Santu Pietru d'Accia	SPA	1
Fangu (Eltru + Saltare)	FAN	2
Piana	PIA	1
Total		220

Genetic polymorphism and population structure

We used MSA 4.05 (Dieringer and Schötterer, 2003), Fstat 2.9.3 (Goudet, 1995), and Arlequin 3.11 (Excoffier et al., 2005) to quantify the genetic diversity of the microsatellite loci for allelic richness, observed and expected heterozygosity, and to test for deviation from Hardy–Weinberg genotype frequency equilibrium (10,000 permutations). We also calculated the Garza–Williamson index, which should be very small in populations having experienced a recent bottleneck and close to 1 in stationary populations (Garza and Williamson, 2001). Presence of null alleles was tested with FreeNa (Chapuis and Estoup, 2007). Smogd 1.2.5 (Crawford, 2010) was used to provide D_{est} , an estimate of population differentiation designed for small sample size. The F_{st} values between pairs of population were calculated in Arlequin (sequential Bonferroni corrections were applied when appropriate). We performed Bayesian analyses using Baps 5.3 (Corander et al., 2008) to determine the optimum group structure from the microsatellite loci. The number of clusters K was set to 2 from 13 and the analysis was conducted at the group level using both mixture model (individuals are assigned to one identify cluster) and admixture model (individuals can be partially assigned to several clusters), following the procedure described in Bailey et al. (2013) for data set with weak population structure. The partitioning of microsatellite diversity within and among the groups found in Baps was quantified by an Amova in Arlequin (significance assessed through 1000 replications). We used Bottleneck 1.2.02 (Cornuet and Luikart, 1997) to assess whether recent declines in population occurred in the nuthatches populations, based on heterozygosity excess test and using a two-phase model (TPM) which allows multiple-step mutations, and 1000 iterations. The three smallest samples (SPA, FAN, PIA, of one or two individuals) were removed for this analysis.

For the mitochondrial data, haplotype and nucleotide diversity was calculated using DnaSP 5.10 (Librado and Rozas, 2009) and a haplotype network was constructed using Network 4.6.1.3 (Bandelt et al., 1999). Signals of population expansion were tested using Harpending's raggedness index r (Schneider and Excoffier, 1999) using DnaSP. We also used Fu's F_s and Tajima's D to test against selective neutrality and population equilibrium (Tajima, 1989). The expansion time t and demographic expansion parameter τ were estimated using Arlequin under a model of pure demographic expansion (Rogers and Harpending, 1992; Schneider and Excoffier, 1999). The validity of the expansion model was tested using the sum of square deviations (SSD) between the observed and expected mismatches as implemented in Arlequin (with 1000 replicates). We used Beast 1.8.0 (Drummond and Rambaut, 2007) to calculate Bayesian Skyline Plots and visualize the demographic expansion through time for the mitochondrial data set. The analyses were run under a population expansion model using the coalescent tree prior, an HKY model of substitution (selected using MrModeltest 2.3; Nylander, 2004), and the number of groups was set to 2. We applied a strict clock with a rate of 10^{-8} substitution/site/year, with a uniform prior for substitution rates varying from 0.0095 to 0.0115, as estimated by Weir and Schluter (2008) for mitochondrial genes in passerines. Independent runs of 20 million generations were performed, with the Markov chain sampled every 1000 generations and a 10% burn-in period. Bayesian Skyline Plots were generated with Tracer 1.4.1 (Rambaut and Drummond, 2007).

Divergence time estimate

We estimated the divergence time and the migration between the two groups found using Baps using the Isolation-with-migration model implemented in Ima2 (Hey and Nielsen, 2007; Hey, 2010) on the complete genetic data set (ND2 sequences and microsatellite loci). This software employs a coalescent-based MCMC simulation to estimate simultaneously these parameters. We conducted several runs of 30 million steps, with a burn-in period of 10^6 steps, and a geometric heating scheme of four to forty chains. Different priors were tested for estimating Θ and t

(from 10 to 100) and similar results were obtained when varying these parameter values. For scaling parameter estimates in demographic units, we specified the same mutation rate of 10^{-8} substitution/site/year for the 546 base pairs ND2 sequence data and let Ima2 calculate mutation rate scalars for the microsatellite loci. Generation time was estimated to 2.5 years (Matthysen, 1998). Runs were monitored by observing effective sample size (ESS) values and by the absence of trends in the parameter plots.

Morphometrics

Among the 220 individuals sampled for genetic analyses, we measured 174 males for wing length (the distance between the carpal joint and tip of the flattened wing), bill length (1, from tip of bill to skull; and 2, from tip of bill to the rear of the nostril), and tarsus length (from notch of the inter-tarsal to the lower edge of the last complete scale). These measurements were compared using ANOVA for the two groups found in Baps analysis to check if they differ in their morphology. We also used principal components analysis (PCA) to summarize patterns of variation in the morphometric data in producing independent composite variables (PC axes). Variables were standardized and the analysis was conducted using the package ggbiplot in R (Vu, 2011).

Results

Five loci (SpuA6, Syl14, Spu4-C6, Pij23, and SpuL6-16) were not polymorphic for the nuthatch and were not included in the analysis. The genetic diversity for the five remaining microsatellite loci in each population is summarized in Table S1 in the supplementary material. The allelic richness varied from 1.3 to 1.7. Deviation from HWE ($\alpha = 0.05$) was found in four cases (LONCA and FRA for locus 2, GUA for Locus 3, VIZ for Locus 1), but null allele frequency estimated by FreeNa for these loci and populations were inferior or equal to 0.2, suggesting that null alleles were uncommon to rare in our dataset. Moreover, pairwise F_{st} estimates with or without correction for the presence of null alleles [the ENA procedure described by Chapuis and Estoup (2007), matrices not shown] did not differ significantly, suggesting that the bias induced by null alleles in the estimation of population differentiation was not significant in our data set. The overall D_{est} was low (0.049) and indicated that less than 5% of the total variation was explained by differences between populations. Pairwise F_{st} were significant after Bonferroni correction for 17 pairs of populations, among which 11 (65%) involved three populations located in the Northern part of the nuthatch range, BON, FRA, and MELA (Table S2 in the supplementary material). These three populations formed one of the two clusters found in Baps as the most likely structure in the microsatellite data set (Fig. 2). The second cluster was composed of all the remaining populations. The partition of diversity within and among these two groups ("NORTH" composed of BON, FRA, and MELA, and "SOUTH" composed of all remaining populations) indicated low but significant genetic subdivisions ($P < 0.001$) in the hierarchical AMOVA, where 3.42% of the total genetic variance was among groups, 2.64% among populations within groups, and 93.94% within populations. No significant heterozygosity excess was detected within populations or within the two groups SOUTH and NORTH (all $P > 0.05$), and the values for the Garza–Williamson index were close to 1 (Table S1), suggesting the absence of detectable recent population bottleneck.

Six haplotypes were found for the ND2 sequences over the 220 individuals, with a haplotype diversity H_d of 0.54, nucleotide diversity Π 0.00106. Two haplotypes were frequent (50.9% and 44%) and shared among all populations and among the NORTH–SOUTH groups (Fig. 3). When all individuals are considered together, the mismatch distribution was unimodal (Fig. 4) with a low value of $\tau = 0.713$ (95% confidence interval 0.056–1.231), suggesting a low population demographic expansion, but this hypothesis was rejected by the significant raggedness index $r = 0.215$ ($P < 0.05$) and $SSD = 0.031$ ($P < 0.05$). Fu's F_s (-0.582)

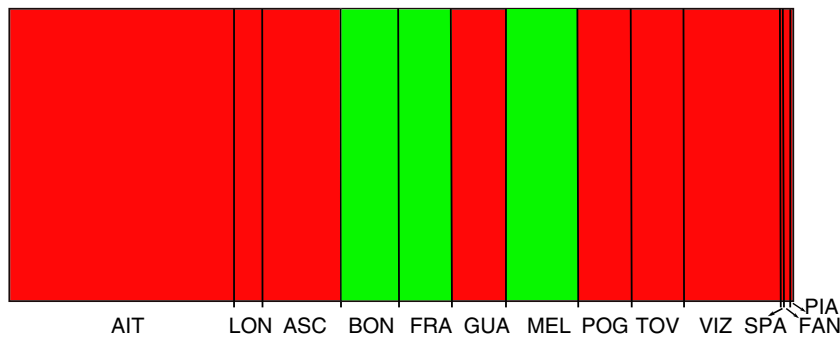


Fig. 2. Individuals bar plots from each geographic location ($n = 13$) corresponding to the optimal clusters ($K = 2$) found using an admixture model and the microsatellite loci. The vertical lines each represent an individual, and colors represent the proportion of that individual assigned to each cluster (green = NORTH, red = SOUTH).

and Tajima's D (-1.47) were negative, also an indication of population expansion, but not significant. When considering the NORTH and SOUTH groups individually, similar results were found (significant raggedness and SDD, negative but not significant F_s and D), with a lower haplotype diversity in the NORTH than in the SOUTH ($Hd_{NORTH} = 0.33$ and $Hd_{SOUTH} = 0.51$). The Bayesian Skyline Plots for the 220 individuals, in accordance of the previous results, showed a low expanding pattern for the nuthatch since the last 7000 yr (Fig. 5).

The different IMA2 runs, based on the data set combining the mitochondrial sequences and the microsatellite loci, yielded similar results and suggested that the two groups NORTH and SOUTH diverged less than 6000 yr (results for the run with the largest ESS, $t = 5187$, 95% confidence interval 1729–19,018), with a migration rate larger from SOUTH to NORTH ($m = 3.15$) than on the opposite direction ($m = 0.15$). The effective population for the ancestral nuthatch population (i.e. before the split in two groups) was estimated at ca. 46,000 individuals ($N_{e-ancestral} = 45,817$, 95% confidence interval 23,341–106,329). The effective population size estimates from the Skyline Plot analysis were consistent with these results but with a large confidence interval (69,000; 95% CI 200,000–9600).

The genetic differentiation found using nuclear markers was not recovered in the morphological characters, as the measurements on wing, bill, and tarsus did not differ significantly for the two groups ($P > 0.1$ in the ANOVAs; mean and standard deviation in mm for NORTH and SOUTH, respectively: Wing 72.1 ± 0.9 and 72.1 ± 1.0 ; Bill1 15.7 ± 1.5 and 15.2 ± 1.1 ; Bill2 12.1 ± 0.6 and 12.1 ± 0.5 ; Tarsus 17.6 ± 0.6 and 17.7 ± 0.5). Results of the PCA show that the two groups are completely mixed in the PCA space and do not differ morphologically (Fig. S1 in the supplementary material).

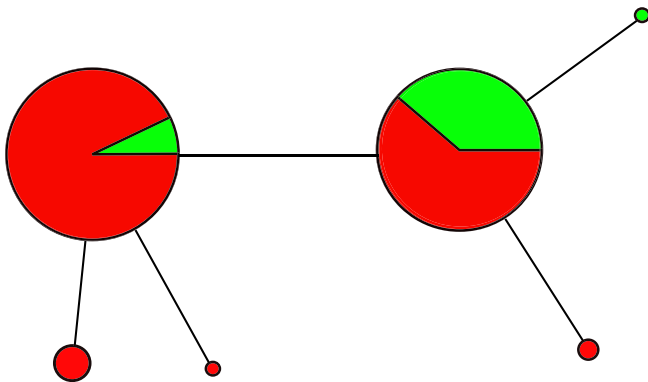


Fig. 3. Maximum parsimony network of mitochondrial ND2 haplotypes. The size of each circle reflects the frequency of the haplotypes. Colors correspond to the groups recovered using the microsatellite data (green = NORTH, red = SOUTH).

Discussion

Corsica, a refugium for forest birds during the last glacial maximum

The lineage that led to the nuthatch diverged from its closest relative, the Chinese nuthatch, at ~ 1 Ma (Pasquet et al., 2014). The ancestral distribution of the nuthatch was probably larger than the sole Corsica Island, in relation with the distribution of the black pine forests (*Pinus nigra* ssp.), assuming that the exclusive preference of the nuthatch for these pines is ancient in the history of the species. Today, black pines are found from the Iberian Peninsula and North Africa to Turkey (Farjon and Filer, 2013), with a mosaic distribution probably modeled by the last glaciations and human actions [see Zaghi (2008) for European directives and recommendations for the management of the black pine forests]. The lario pine diverged from the Italian populations at about 100 ka (Afzal-Rafii and Dodd, 2007). In the absence of evidence of other food resources in the past, the ancestral Eurasian nuthatch presumably colonized Corsica only after the pine's arrival. After the establishment of both the pine and the nuthatch on the island, several glacial episodes occurred in Europe, culminating with the last glacial maximum (LGM, 24.0–15.0 ka) (Frenzel, 1992). These glacial episodes provoked the contraction of the forests that were reduced to refugia in southern Europe, where forest bird populations could survive in lower numbers (Newton, 2003). In Corsica, pollen and sub-fossil charcoals studies indicated that *Artemisia* steppes replaced the forests above the altitude of 1300 m during the LGM (Reille, 1975; Reille

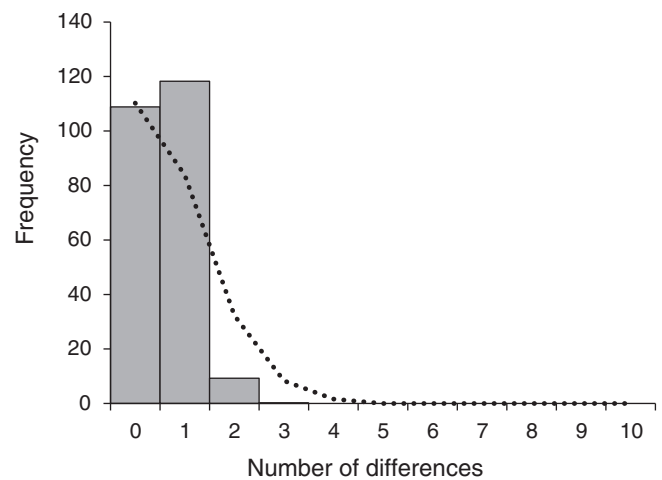


Fig. 4. Mismatch distributions for the Corsican nuthatch mitochondrial ND2 haplotypes. Histogram: observed distribution. Dotted line: mismatch distribution expected under a pure demographic expansion model.

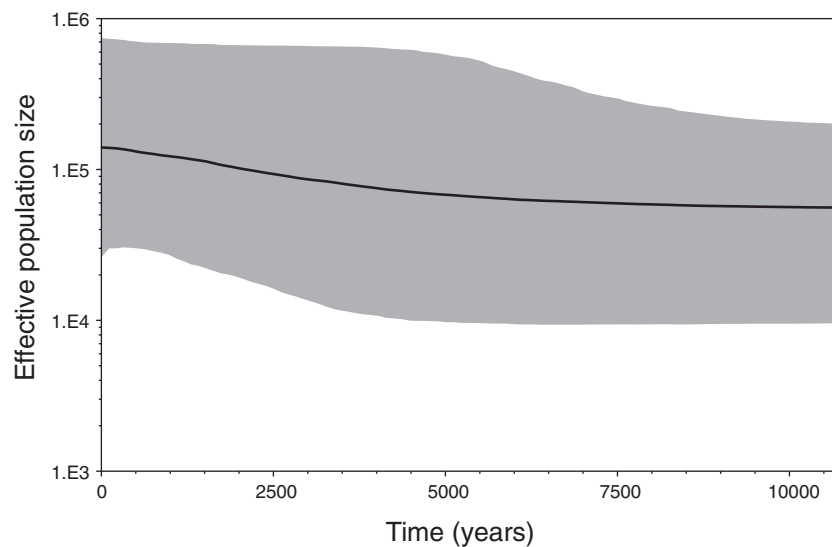


Fig. 5. Bayesian Skyline Plots for the mitochondrial data set.

et al., 1997; Leys et al., 2014). But the ancient genetic differentiation of the pine from its Italian counterpart, and its presence in the interior of the island since at least 13.2 ka (Leys et al., 2014) confirm the persistence of the pine through the glacial cycles, especially on the coastal areas, probably facilitated by the lowering of the sea level (Lambeck et al., 2004). Although we cannot reject the hypothesis of a recent, post-LGM dispersal of the nuthatch to Corsica, the continuous presence of the pine in Corsica is consistent with the most parsimonious hypothesis of co-evolution in the island, suggesting that the nuthatch survived also *in situ* the Late Pleistocene glaciations.

Molecular studies also support this hypothesis in showing that Corsica has been a glacial refugium for other forest birds during the Pleistocene. First, Pons et al. (2015) showed that the Eurasian treecreeper of Corsica, a taxon currently recognized as a subspecies (*Certhia familiaris corsa*), belongs to a lineage that disappeared from the European mainland during a glacial period and persisted only in the Caucasus region and in Corsica. The rest of Western Europe, including nearby Italy, was later colonized by another lineage. Their calibrated phylogeny suggested that the colonization of Corsica by treecreepers occurred, like the nuthatch, during the second half of the Pleistocene (~ 1 Ma). This implies that treecreepers were present in Corsica during the Pleistocene glacial periods. Similarly, the blue tit (*Cyanistes caeruleus*) populations persisted in Corsica during the LGM; surprisingly, populations from Spain recolonized Italy after the last glacial episode, and not the close-by Corsican blue tit (Kvist et al., 2004). Treecreepers and blue tits are not strictly associated with pines but inhabit also deciduous forests, suggesting that these forests sustained the Pleistocene glacial periods in the island as well. Globally, the situation in Corsica contrasts with continental borderlands, for instance in the Alps, Appennino, and Abruzzo, where many forested species had not persisted *in situ* and were forced to find refuge in the peninsulas of Southern Europe (Taberlet et al., 1998; Brito, 2005; Pons et al., 2011). Prodon et al. (2002) showed that the repetitions of the climatic cycles have had an influence on the ecology of the resident bird species by favoring a broadening of their habitat-niche.

Forest expansion during warmer periods

After the LGM, and particularly during the warmer “Atlantic” period (9000–5500 BC; Nesje and Dahl, 1993; Wanner et al., 2008), evidence from pollen and charcoals studies indicated a large expansion of the pine all over the island, from the coast up to at least 2050 m asl, often mixed with deciduous trees like white oaks (*Quercus* sp.) (Reille,

1975, 1977; Thion, 1998). Increase of its potential habitat likely resulted in the expansion of the nuthatch population over the island: the demographic results found based on the mitochondrial data (Fig. 5) could correspond to the increase of pine forests during the warmer periods of the Holocene, although the not significant statistical indices suggest that this growth was limited. Our estimate of the ancestral effective population size for nuthatches found in IMA2 using genetic data (46,000 individuals) is on the same degree of magnitude as a rough estimate of the ancestral population size extrapolated from the maximal surface of pure pine forests and the actual density of birds (several tens of thousands of couples: Thibault, 2006). This estimate, however, depends on the proportion of deciduous species mixed with the pine stands after the LGM, which is unknown. Today the nuthatches do not tolerate mixed stands where the proportion of deciduous trees (mainly beeches *Fagus sylvatica*) exceeds 50% (Villard et al., 2014). They can also occupy pure Maritime Pine stands at low elevations (Thibault et al., 2011), relying only on insects as they cannot eat the too large and hard seeds: clearly this habitat, which is recent (Carcaillet et al., 1997; see below) and has been favored by anthropic actions, is suboptimal for the survival of the nuthatch.

Anthropic forest regression and consequences for the nuthatch populations

The two different groups of nuthatch revealed by the nuclear markers diverged recently, long after the LGM, and they should be then interpreted as the result of habitat modifications by humans whose first permanent settlements in the island dated back to 9.0 ka (Costa, 2004). Fires and deforestation for agriculture and livestock entailed, first, a reduction of the native forests since 6.0–5.0 ka (Weiss, 1976, 1992; Costa, 2004), and second, a turnover of the species observed in pollen sediments: birches (*Betula pendula*), firs (*Abies alba*), and beeches replaced locally the laricio pine (Reille, 1975; Thion, 1998). Between 2.5 and 1.0 ka, the laricio pine disappeared completely from the littoral regions, replaced mainly by the maritime pine (*Pinus pinaster*) and the holm oak (*Quercus ilex*) (Reille, 1992; Carcaillet et al., 1997; Thion, 2003). The maritime pine, well adapted to fires (Pimont et al., 2011), constitutes today most of the coniferous stands, up to 1000–1200 m inland (ONF, 2006), in place of the original laricio pine forests that are more fire-sensitive and which range is restricted to Supramediterranean and Montane vegetation zones (Gamisans, 1991). Thus the nuthatch suffered a reduction and a fragmentation of its habitat since the Neolithic. In addition to forest clearing for agriculture, the search of large trunks for shipbuilding during the Republic of Genova

since the 14th century (Rota and Cancellieri, 2001; Fontana, 2004) and under the French rule until the 19th century led to a substantial reduction of the old pine stands that are the optimal habitat of the nuthatch (Thibault et al., 2006). The group SOUTH comprises most of the populations included in this study and shows no evidence of structure; the connectivity between its populations was maintained because the forest patches are not separated by high mountain chains (Fig. 6). In contrast, the group NORTH is composed of a few small forest stands isolated from the rest of the island by areas without pine forest and by rugged mountainous terrains 2000 m. This isolation clearly results from the anthropization of the landscape (replacement of pines by pastures in high altitudes, by holm oak stands in low altitudes). Furthermore, the extreme sedentariness of the nuthatches facilitated the isolation of the two groups (Fig. 6). The molecular analyses suggest that the larger population from the south migrated more often to the north than the reverse. The lack of morphometric differences results probably from the recent divergence of the two groups and from the fact that they occupy similar habitats.

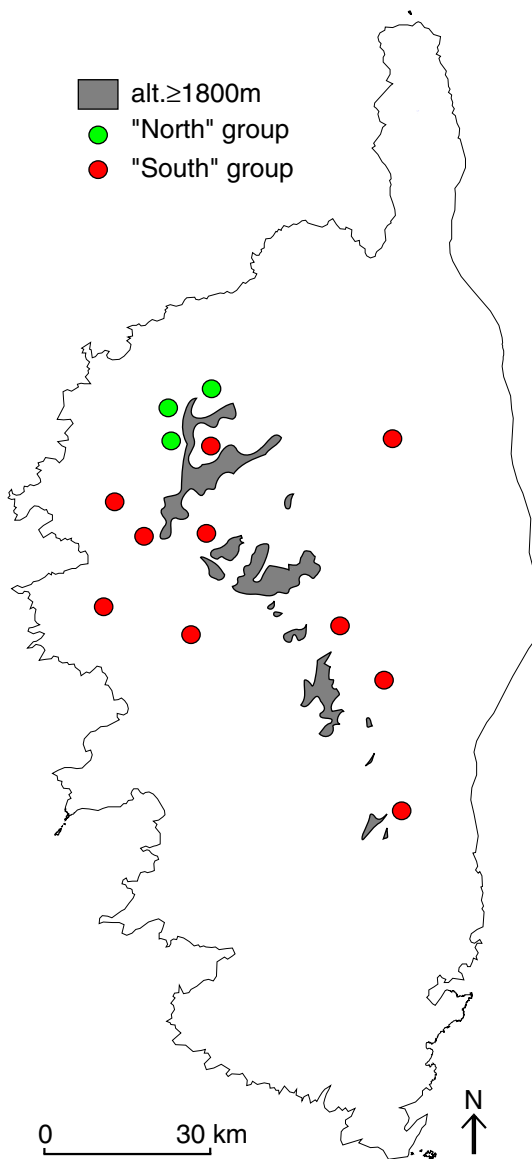


Fig. 6. In gray areas with an altitude equal and superior to 1800 m, the upper limit of the forests; this corresponds to barriers for the dispersal of nuthatches. The dots' colors correspond to the groups recovered using the microsatellite data (green = NORTH, red = SOUTH).

Interestingly, several phylogenetic studies on endemic Corsican animals found distinct evolutionary lineages in the northern part of the island, although with older divergence time estimates (Pliocene) for most species: newt *Euproctus montanus* (Bisconti et al., 2013), lizard *Archeolacerta bebrigi* (Salvi et al., 2010), woodlouse *Helleria brevicornis* (Gentile et al., 2010), and land snail *Solatopupa guidoni* (Ketmaier et al., 2010). Only the Corsican fire salamander *Salamandra corsica* presented similar temporal pattern, with a recolonization of the northern range of the island that occurred during the Pleistocene (Salvi et al., 2015). This suggests that the topography of the northern part of the island and its mountain chains promote the isolation of the populations, irrespectively of the different climatic or ecologic events responsible for the fragmentation process. For birds, the only other phylogenetic study within Corsica found a genetic differentiation in the blue tit populations associated with different habitats, and not with geographic barriers (Porlier et al., 2012). Further work is necessary to look for genetic structure within other endemic Corsican birds, particularly the forest species like the treecreeper, for which the influence of Pleistocene climatic variation has already been demonstrated at the continental scale (Pons et al., 2015).

Conclusions

Our scenario clearly relies on a niche conservatism hypothesis, in which the nuthatch's ecological preferences, closely associated with the laricio pine, actually reflect the ecology of this bird in the past. Although the presence of nuthatches in suboptimal habitats today provides evidence for a relative plasticity in the nuthatch's ecology, these populations remain marginal and in low density, despite the increase of maritime pine stands favored by fires. Thus we suggest, on the basis of the most parsimonious scenario obtained from this study, that the nuthatch should be an old element of the Corsican avifauna that endured the late Pleistocene and Holocene climatic variations and their subsequent cycles of forest contractions/expansions. The hypothesis cannot be dismissed that the range of the nuthatch has been fragmented during the LGM, and that the differentiation of the population fragments was erased during the post-LGM episodes of forest expansion that have reunited all the populations. However, our results suggest that the recent anthropization of the landscape resulted in the isolation of a cluster of populations in the northern part of the island. No bottlenecks were detected, suggesting that this cluster did not experience any loss of genetic diversity that could have resulted from a decline in its effective population size. The recent isolation of this northern group reveals, however, a reduced gene flow within the island.

Interestingly, the nuthatch did not colonize the close pine forests of Southern Italy when its number was increasing after the last glacial episode, i.e., a "reverse colonization," from island to continent. It has been shown, however, that in other parts of the world, an insular status did not prevent bird species to colonize continents, or islands as large as Australia, from small and remote islands (Filardi and Moyle, 2005; Bellemain and Ricklefs, 2008; Cibois et al., 2011).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yqres.2016.01.002>.

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