

## HISTORICAL DIVERSIFICATION OF MIGRATION PATTERNS IN A PASSERINE BIRD

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**Abstract.**—Migratory strategies of birds require complex orientation mechanisms, morphological adaptations, and life-history adjustments. From an evolutionary perspective, it is important to know how fast this complex combination of traits can evolve. We analyzed mitochondrial control-region DNA sequences in 241 blackcaps (*Sylvia atricapilla*) from 12 populations with different migratory behaviors. The sample included sedentary populations in Europe and Atlantic archipelagos and migratory populations with different distances of migration, from regional to intercontinental migrations, and different heading directions (due to a migratory divide in central Europe). There was no genetic structure between migratory and sedentary populations, or among populations from different biogeographic areas (Atlantic islands, the Iberian Peninsula, or the continent), however we found evidence of a genetic structure when comparing populations located on either side of the migratory divide. These findings support an independent evolution of highly divergent migratory strategies in blackcaps, occurring after a postglacial colonization of the continent along western and eastern routes. Accordingly, mismatch-distribution analyses suggested an expansion of blackcaps from a very small population size, and time estimates dated such an expansion during the last postglacial period. However, the populations in Gibraltar, located in a putative Mediterranean refuge, appeared to be independent of these processes, showing evidence of restricted gene flow with other populations and demonstrating insignificant historical changes in effective population size. Our results show that the interruption of gene flow between migratory and sedentary populations is not necessary for the maintenance of such a polymorphism, and that even the most divergent migratory strategies of a bird species are susceptible to evolution in response to historical environmental changes.

**Key words.**—Adaptive divergence, environmental change, evolution of migration, mitochondrial control region, population genetic structure, mismatch distribution, postglacial expansion, *Sylvia atricapilla*.

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The capacity of organisms to rapidly adapt to environmental changes, and the historical and ecological constraints on such adaptations are central to evolutionary theory (Orr and Smith 1998; Lenormand 2002). Behavior, morphology, and physiology can evolve rapidly when environments change (Orr and Smith 1998), however rapid adaptive evolution may be constrained by different mechanisms, for example if genetic correlations between different traits impose nonadaptive correlated responses of traits that are not directly selected (Pulido and Berthold 2003). Rapid environmental changes have occurred historically, for example, during alternating glacial and postglacial periods (Hewitt 2000), and still faster changes will surely take place due to global warming (Both and Visser 2001). Understanding the potential for rapid evolution of complex phenotypic traits is important if we are to anticipate population responses to these changes.

Among bird species, migratory behavior does not seem to be evolutionarily constrained as both residency and long distance migration seem to evolve repeatedly (Helbig 2003). However, within species in ecological time, there is a general concern about the ability of migratory birds to cope with the environmental changes expected from global warming, because the migratory program in some species shows little potential for rapid evolutionary change (Both and Visser 2001). Unravelling the tempo of diversification of extant migratory patterns of birds might help us to resolve this uncertainty. Due to differences in habitat seasonality associated with latitude and elevation, birds show a great diversity of

migratory strategies, both among and within species (Herrera 1978; Newton and Dale 1996; Tellería et al. 2001). For example, a single Palearctic bird species may include sedentary populations, intercontinental migrants, and all intermediate degrees of partial and/or short-distance migration (e.g., Cramp 1992).

The evolution of different migratory strategies involves changes in a complex combination of behavioral, ecological, and life-history traits (Berthold et al. 2003). Migrant birds require orientation mechanisms to reach their final destination, sometimes including innate adjustments of direction en route to fit the best itinerary in terms of time or energy (Wiltschko and Wiltschko 2003). Storage and administration of energy necessary for long-distance flight depends on physiological adaptations including seasonal fattening, changes in the metabolism of proteins and fatty acids, and organ plasticity (Piersma 1998). Morphological adaptations may also evolve to reduce the cost of migratory flight, thus migratory birds often differ from their sedentary conspecifics in having longer and more pointed wings, a shorter tail and, sometimes, a reduced body size (Winkler and Leisler 1992; Tellería et al. 2001). In addition, migratory birds need to adjust their life cycles to complete their breeding season, molt, and other activities between migration periods (Gwinner 1996). Concomitant life-history adjustments include increased fecundity and reduced survival, due to a combination of reproductive benefits of exploiting seasonal habitats and mortality costs associated with migration (Pérez-Tris and Tellería 2002a).

Different features of migratory strategies, such as timing, orientation, or flight distance, have been observed to evolve rapidly in nature (Berthold et al. 1992; Able and Belthoff 1998; Pulido et al. 2001), and birds can also acquire or lose migratory activity in a few generations in selection experiments (Berthold 1996). But other, more complex traits related to migration, such as morphological or physiological adaptations, might be less susceptible to rapid evolutionary change, because behavior could be more prone to rapid adaptive change than other parts of the phenotype (Wilson 1975). This introduces an important uncertainty regarding the age of divergence of extant migratory behaviors of some species. In the Palearctic, for example, many bird species colonized northern areas when these became available with the retreat of glaciations, around 18,000 years ago (Taberlet et al. 1998), but we do not know whether these species acquired—or regained—their diverse migratory behaviors during the colonization process (as resident birds expanded towards more seasonal areas from southern refugia), or whether such a diversity had already evolved before and just persisted in refuge areas during the glaciations.

Rapidly evolving neutral genetic markers may help us to unravel how fast the complex combinations of traits underlying different migratory strategies can evolve (Avise et al. 1987; Baker and Marshall 1997). We studied mitochondrial control-region DNA sequence variation among populations of a typical temperate migrant passerine, the blackcap *Sylvia atricapilla*. This species shows extensive differences in migratory behavior across its range, accompanied by parallel differences in physiology, morphology and life-history traits, which together define highly divergent migratory strategies (Cramp 1992). In addition, the blackcap has become the model species in laboratory studies of migration physiology and genetics, and thus the mechanisms of control and the adaptability of many aspects of its migration are well known (Berthold 1996, Pulido and Berthold 2003). We studied whether migratory strategies of blackcaps diverged early in the species' history or, on the contrary, if migratory behavior was recently acquired or lost among blackcap lineages. In particular, we analyzed (1) whether differences between migratory and sedentary blackcap populations sampled at different spatial scales contribute to molecular variance in the mitochondrial control region; (2) how the effective population size of migratory and sedentary populations has changed in historical time (i.e., whether it has remained constant or if there is any evidence of population expansion, and when these possible demographic events may have occurred); and (3) when blackcap populations with different migratory behaviors diverged from each other and how much gene flow they maintain.

## METHODS

### *Species, Study Area, and Sampling Methods*

The blackcap is a widespread forest passerine in Europe, showing the highest diversity of migratory behaviors described so far within a species. Blackcaps breeding in Scandinavia travel long distances to winter in sub-Saharan Africa, contrasting with the completely sedentary populations breeding in southern Mediterranean areas and Atlantic islands. In

addition, the species includes partially to completely migratory populations in southern and central Europe, which show broad ranges of migratory distances and directions due to the existence of migratory divides (Cramp 1992; Berthold 1996). The most important of these divides, roughly located at 12–13°E, separates western and eastern migrants in central Europe (Cramp 1992; Fig. 1).

As in most temperate birds, migratory propensity has an important geographic component in blackcaps: in general, populations are migratory in the north and sedentary in the south of the range. This general association between latitude and migratory behavior makes it difficult to resolve whether geographic distance or migratory behavior per se is responsible for genetic divergence between migratory and sedentary populations sampled on a latitudinal gradient. To avoid this problem, and also to study blackcap migratory strategies that are as divergent as possible, we sampled migratory and sedentary populations at two geographic scales (Fig. 1).

At a small scale, we studied blackcaps in the Iberian Peninsula. We sampled two migratory populations breeding in northern highlands, one in northern Spain, at the northern edge of the Spanish plateau (Álava; 42°55'N, 2°29'W), and another one in the central Spanish mountains (Guadarrama; 40°54'N, 3°53'W). We also studied two sedentary populations in the Iberian lowlands: one on the north side of the Strait of Gibraltar, at the southernmost edge of Spain (Tarifa-Los Barrios; 36°01'N, 5°36'W), and the other along the coast of Portugal (Lisbon; 38°43'N, 9°08'W). Although distance should not be an important barrier to gene flow among Iberian blackcaps, these populations show strong differences in morphology related to migratory behavior (Tellería and Carbonell 1999), and they also differ in clutch size and longevity (Pérez-Tris and Tellería 2002a), which make them ideal for studying the correlation between differences in migratory strategy and genetic variation independent of geographic location.

At a larger scale, we analyzed eight more populations representing the whole range of variation in migratory behaviors of blackcaps. These included two populations located at the eastern side of the migratory divide in Europe, one in southern Sweden (Malmö; 55°36'N, 13°00'E), and the other in southeastern Austria (Bruck an der Leitha; 48°01'N, 16°48'E). Also, we studied three populations of western migrants from central Europe, sampled in the UK (Ivinghoe; 51°45'N, 0°48'W), Belgium (Quievrain; 50°24'N, 3°41'E), and France (Dijon; 47°19'N, 5°01'E). Finally, extremely isolated and sedentary blackcaps were sampled in the Atlantic archipelagos of Madeira (Madeira; 32°38'N, 16°54'W) and the Canary Islands (Gran Canaria; 28°06'N, 15°24'W). All birds, including full grown individuals and unrelated nestlings, were sampled during the breeding season (June–August, from 1991 to 2003) to avoid including active migrants. DNA was obtained mostly from blood samples (ca. 50 µl) collected from the brachial vein and stored in blood-preservation buffer, or less frequently from feathers that had been plucked from the tail or inner wing.

### *DNA Analyses*

Total genomic DNA was extracted from blood or feather samples using the standard proteinase k phenol/chloroform

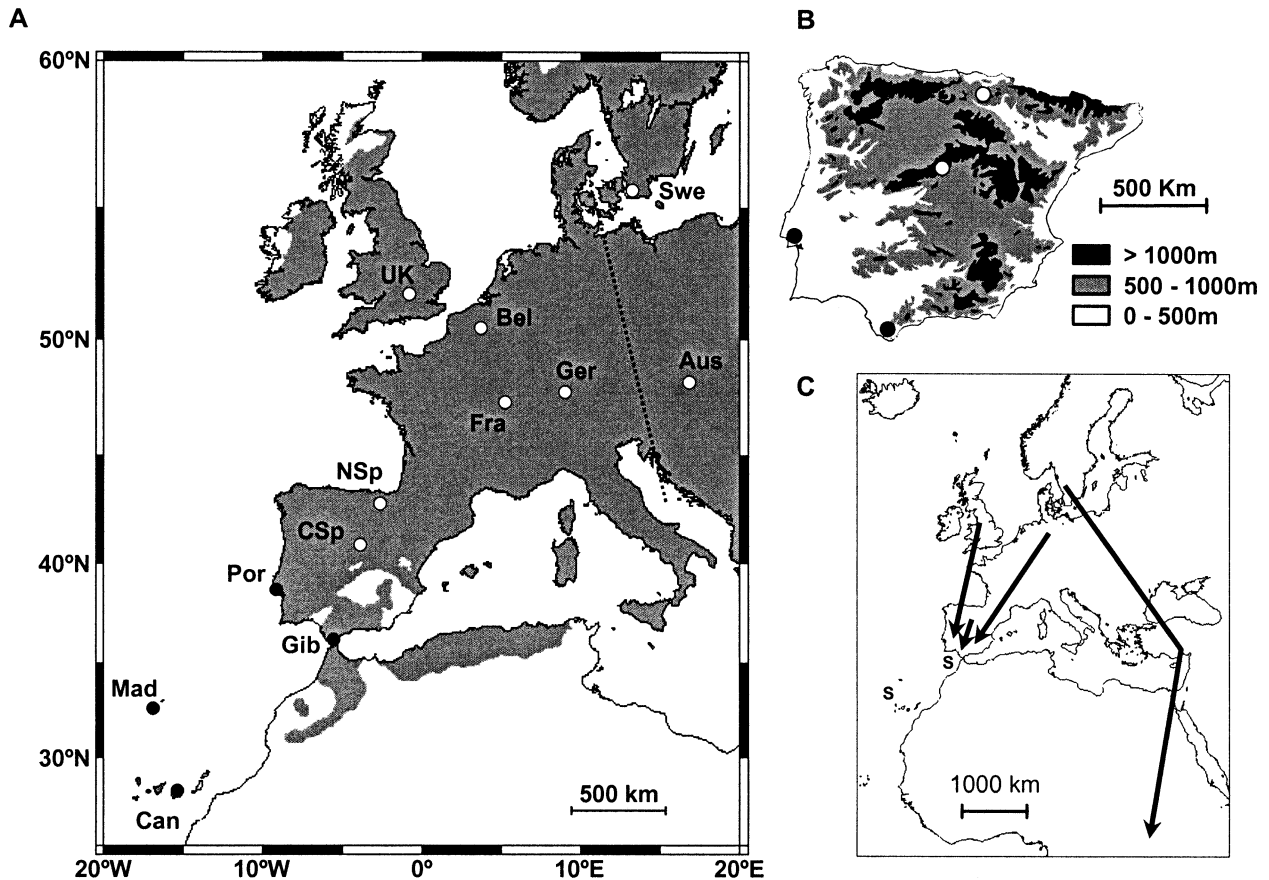


FIG. 1. (A) Breeding distribution of western Palearctic blackcaps (shaded area), location of the migratory divide in central Europe (dashed line), and localities sampled in this study. Open and filled circles denote migratory and sedentary populations, respectively. Populations are labeled as UK (United Kingdom), Fra (France), Bel (Belgium), Ger (Germany), NSp (Northern Spain), CSp (Central Spain), Por (Portugal), Gib (Gibraltar), Swe (Sweden), Aus (Austria), Mad (Madeira), and Can (Canary Islands). (B) Detailed location of sites sampled along the altitudinal gradient in Iberia. (C) The major migratory patterns of the populations studied (S: sedentary). Maps in A and C based on Cramp (1992).

technique, precipitation with 4M  $\text{NH}_4\text{Ac}$  and ethanol, or boiling. Genomic DNA extracts were diluted to a working concentration of 25 ng/ $\mu\text{l}$ .

We used the primers L437 and H1248 (Tarr 1995) to amplify a fragment of about 750 base pairs (bp) from the central domain to the 3' end of the control region, including the 5' end of the  $t\text{RNA}^{\text{Phe}}$  gene. PCR reactions were set up in 25  $\mu\text{l}$  total volumes and included 25 ng of template DNA, 1  $\times$  PCR buffer (Perkin Elmer), 0.125 mM of each nucleotide, 0.6 mM of each primer, 1.1 mM  $\text{MgCl}_2$ , and 0.5 units of AmpliTaq DNA polymerase (Perkin Elmer). Amplifications started with 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 50°C and 60 sec at 72°C. Each reaction was terminated by a 10-min extension at 72°C. We evaluated 2.5  $\mu\text{l}$  of each reaction on a 2% agarose gel stained with ethidium bromide and using 0.5  $\times$  TBE buffer. The remaining was precipitated by adding 11  $\mu\text{l}$  of 8 M  $\text{NH}_4\text{Ac}$  and 33  $\mu\text{l}$  ethanol, and resuspended in 15–20  $\mu\text{l}$  water. We used 2  $\mu\text{l}$  for sequencing 503 nucleotides in the control region from the 5' end of the PCR fragments (the  $t\text{RNA}^{\text{Phe}}$  gene was excluded, and the 3' end of the control region was not considered because its sequence reading was often poor), using

a dye-terminator AmpliCycle sequencing kit (Perkin Elmer) and an ABI PRISM™ 310 (Perkin Elmer, Boston, MA) sequencing robot, following manufacturer's recommendations.

#### Genetic Diversity and Structure

Sequences were manually aligned and edited using BioEdit (Hall 1999). Standard indices of haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversity, and Tajima's  $D$  testing for selective neutrality (Tajima 1989) were computed using DnaSP 2.52 (Rozas and Rozas 1997). We used the program Arlequin (Schneider et al. 2000) to partition out the total genetic variation into different components by means of analysis of molecular variance (AMOVA; Excoffier et al. 1992) taking into account the number of substitutions between haplotypes and using Kimura two-parameter distances under a gamma distribution with  $\alpha = 0.02$ , as estimated from the data using the program Tree-Puzzle 5.0 (Schmidt et al. 2002). We first examined overall differences among populations, and then tested for genetic structure in relation to differentiation between migratory and sedentary populations, different geographic areas (Atlantic Islands, Iberian Peninsula, and the rest of the con-



continent), and sides of the migratory divide. This analysis allowed us to partition genetic variance into components associated with within-population differences (Vc), differences among populations within the groups compared (Vb), and differences among groups (Va) (Excoffier et al. 1992). We then computed fixation indices corresponding to each component (as defined in terms of coalescent times; Schneider et al. 2000), the significance of which was tested by 5000 permutations of haplotypes among populations among groups (Vc and  $\Phi_{ST}$ ), haplotypes among populations within groups (Vb and  $\Phi_{SC}$ ), or populations among groups (Va and  $\Phi_{CT}$ ) (Excoffier et al. 1992).

Because we studied populations showing different degrees of geographic isolation, we controlled for possible isolation-by-distance effects on molecular variation. To do so, we conducted a Mantel test for correlation between the matrices of interpopulation  $\Phi_{ST}$  (estimated from Kimura two-parameter genetic distances between haplotypes,  $\alpha = 0.02$ ) and geographic distances between study sites, as implemented in Arlequin (Schneider et al. 2000). Geographic distances were computed using the program “geod,” a part of the “PROJ” system available from the U.S. Geological Survey at <http://www.remotesensing.org/proj/>. Given that the island populations could be highly influential in this correlation (because they are located at a long distance to other populations but also highly isolated by physical barriers), we repeated the analysis using continental populations only. The phylogenetic relationships among unique mtDNA haplotypes were inferred using the neighbor-joining method as implemented in MEGA 2.1 (Kumar et al. 2001), using Kimura two-parameter distances with  $\alpha = 0.02$  (Schneider et al. 2000). Alignment gaps due to indels were deleted pairwise in these analyses.

#### Historical Population-Size Changes

We tested for possible changes in long-term effective population size by studying the frequency distribution of pairwise differences between haplotypes, also known as mismatch distribution (Rogers 1995). In a population at equilibrium between the effects of mutation and genetic drift,  $\theta$  equals  $2uN$  where  $\theta$  is the mean number of pairwise differences,  $u$  is the mutation rate of the sequence (number of mutations per sequence per generation), and  $N$  is the female effective population size. If the population has maintained a long-term constant population size, the mismatch distribution is expected to follow (Watterson 1975)

$$F_i = \frac{\theta^i}{(\theta + 1)^{i+1}}$$

where  $F_i$  is the proportion of pairs of haplotypes differing by  $i$  substitutions and  $\theta$  is estimated from the data as the observed mean number of pairwise differences (Rogers and Harpending 1992). Rogers and Harpending (1992) also calculated the expected distribution of  $F_i$  assuming that an initial population of female size  $N_0$  at equilibrium suddenly increases (or shrinks) to a female population size  $N_1$ , which is sampled  $t$  generations later. Although this model is very convenient in that it reduces population history to just three parameters ( $N_0$ ,  $N_1$ , and  $t$ ), it has the problem that the effect

of each is confounded with  $u$ , the sum of per nucleotide mutation rates in the region of DNA studied. Thus, the actual mutation rate per nucleotide and generation ( $\mu$ ) has to be known in order to estimate the time since population-size change ( $t$ ), assuming that  $\tau = 2ut$  ( $\tau$  measures time in mutational events), and  $u = \mu L$ , where  $L$  is the length of the sequence of DNA analyzed (Rogers and Harpending 1992). However, the rate of molecular evolution of the avian control region seems highly variable among species (Ruokonen and Kvist 2002). Comparative studies have estimated mutation rates ranging between 0.01—equalling the rate of divergence of the cytochrome *b* gene—and 0.1 substitutions per site per million years (s/s/Myr) (Baker and Marshall 1997). However, a more direct estimate of the intraspecific rate of evolution of the control region in humans, obtained using large pedigrees of mitochondrial lineages (Sigurðardóttir et al. 2000), suggests a rate of evolution of the control region of 0.32 s/s/Myr, much higher than estimates based on avian comparative studies (Baker and Marshall 1997). In turn, comparative studies are likely to underestimate mutation rates (Sigurðardóttir et al. 2000), and we do not know whether birds share the higher mutation rate observed in humans. To take into account this uncertainty, we report our results calculated for low (0.1 s/s/Myr), intermediate (0.2 s/s/Myr) and high (0.3 s/s/Myr) substitution rates, ranging between the maximum rates estimated in comparative studies and the value estimated in humans (Baker and Marshall 1997; Sigurðardóttir et al. 2000).

We used the program DnaSP 2.52 (Rozas and Rozas 1997) to compute  $\theta$  and  $\tau$ , and chi-square statistics to test whether mismatch distributions fit better a model of constant population size or one of population-size changes (also known as the sudden expansion model; Rogers 1995). We also estimated population size before and after expansion assuming that  $\theta_0 = 2uN_0$  and  $\theta_1 = (v - m)^{1/2}$ , where  $m$  is the mean and  $v$  the variance of the observed pairwise number of differences, and considering that the intercept of the mismatch distribution ( $F_0$ ) approximately equals  $1/(1 + \theta_1)$  (Rogers and Harpending 1992). In our calculations, we assumed a generation time of two years for the blackcap, based on an age at maturity of one year and an average annual mortality of around 50% (Cramp 1992).

#### Between-Population Isolation and Migration

The observed patterns of between-population genetic divergence in blackcaps could be due to differences in divergence time, rate of migration, or both. Nielsen and Wakeley (2001) developed a method to jointly obtain nonequilibrium coalescent estimates of divergence time and migration rate between pairs of populations, using the variance in pairwise differences between DNA sequences. Their model considers three parameters:  $N$ ,  $t$ , and  $m$ .  $N$  is the effective population size, which is assumed to be equal for both populations,  $t$  is the divergence time in generations, and  $m$  is the proportion of each population that is replaced by migrants from the other population each generation (gene flow is assumed to be symmetrical). We used the program MDIV (Nielsen and Wakeley 2001), which applies a Metropolis-Hastings Markov chain Monte Carlo method to estimate the parameters  $\theta$  ( $\theta = 2uN$ ),

T and M, the latter being scaled estimates of divergence time and migration rate, respectively. The method assumes a uniform prior distribution of  $\theta$ , M, and T, and derives posterior probability distributions within parameter spaces that are constrained a priori. We constrained parameters within the ranges  $\theta \in [0, \infty]$ ,  $M \in [0, 30]$ , and  $T \in [0, 10]$ , which should encompass most biologically relevant values (Nielsen and Wakeley 2001). Posterior distributions of  $\theta$ , M, and T are obtained by sampling gene genealogies in proportion to the product of their likelihood with respect to the data, and their prior probability with respect to a coalescent distribution, on a single Markov chain with state space defined by all possible genealogies (algorithms are detailed in Nielsen and Wakeley 2001). The length of the Markov chain was 2,000,000 cycles, preceded by 500,000 cycles as a “burn-in” period to avoid dependence on initial conditions (Nielsen and Wakeley 2001).

Once  $\theta$ , M, and T were thus estimated, we worked out divergence times ( $t$ , in generations) and migration rates ( $m$ ) considering that  $T = t/2N$  and  $M = 2Nm$  (Nielsen and Wakeley 2001). As in our analysis of population-size changes, we report nonequilibrium estimates of divergence times and migration rates assuming low (0.1 s/s/Myr), mid (0.2 s/s/Myr), and high (0.3 s/s/Myr) rates of evolution of the control region (Baker and Marshall 1997; Sigurðardóttir et al. 2000). Given that this method uses a nonequilibrium coalescent model of population divergence (which should produce more realistic estimates of gene flow than equilibrium methods applied to recently diverged populations; Nielsen and Wakeley 2001), it also allowed us to evaluate whether our previous analyses of population expansion and isolation could be affected by violation of the assumption that populations are at equilibrium.

## RESULTS

A total of 40 different haplotypes were identified among 241 blackcaps (Table 1), of which three were relatively common (haplotypes 1, 2, and 4) and 22 were found in single individuals only (Table 2). Haplotype 10 was very different from all others (Table 1), showing a 2% divergence from haplotype 1 (including indels as mutations). We sequenced 319 base pairs of the cytochrome *b* of the individual with haplotype 10 and another five individuals representing unique control region haplotypes, and found a similar result for this gene (2.5% sequence divergence between this individual and the others, among which only two haplotypes differing in a single base were identified). We concluded that this individual either had an ancient mitochondrial variant preserved in the central Spanish population, or, that it had a nuclear mtDNA copy (a NUMT) that was exclusively amplified in favor of the true mtDNA in both experiments (independent PCR of partial cytochrome *b* and the control region). We think the latter explanation is highly unlikely, but nonetheless we decided to exclude this individual from our analyses as it represents an outlier in the statistical distribution. Although including this individual would not change our results for population genetic structure or isolation-by-distance tests (results not shown), the mismatch distribution would obvi-

ously be distorted due to the many pairwise differences accounted for by a single haplotype (Rogers 1995).

The remaining 39 haplotypes were distinguishable by 28 polymorphic sites of which 18 were transitions, three were transversions and seven were indels (Table 1). Both haplotype diversity and nucleotide diversity were similar in migratory and sedentary populations, although Gibraltar and Madeira showed the lowest values for both parameters (Fig. 2). In the case of Gibraltar, this effect was explained by a large frequency of the haplotype 2 (found in more than half of the individuals; Table 2), as this population showed higher nucleotide diversity than expected from haplotype diversity, compared to other populations (Fig. 2). All populations met the assumption of selective neutrality, as shown by non-significant Tajima's *D* statistics (all with  $P > 0.10$ ).

### Genetic Structure

Differences among blackcap populations accounted for a significant 3.6% of total molecular variance (Table 3). Accordingly, hierarchical analyses testing for differences between migratory and sedentary blackcaps, or among different biogeographic areas (Atlantic Islands, Iberian Peninsula, and the continent) showed significant differences, of the same magnitude, among populations within groups (Table 3). However, none of these analyses revealed any significant difference between migratory and sedentary populations, or among the three biogeographic areas compared, such effects explaining less than 0.3% of molecular variance in both cases (Table 3). In contrast, a test for genetic structure associated to the migratory divide in Europe (excluding island populations), showed significant differentiation between western and eastern populations, leaving less than 2% of variance to be explained by among-population differences within groups, which was not statistically significant (Table 3). Restricted gene flow on the continent was also revealed by a Mantel test, showing a correlation between  $\Phi_{ST}$  and geographic distance matrices that was significantly higher than expected through random effects ( $r = 0.33$ ,  $P = 0.033$  after 5000 permutations of matrix elements). When the island populations were incorporated in this analysis, the correlation was weaker and not significant ( $r = 0.28$ ,  $P = 0.058$ ). Isolation-by-distance in the continent was due to the differentiation between western and eastern populations. Thus, the correlation between genetic and geographic distances was not significant when only western continental populations were considered (excluding Sweden and Austria;  $r = 0.23$ ,  $P = 0.17$ ), and this effect was still weaker when island populations were added to the analysis ( $r = 0.07$ ,  $P = 0.35$ ).

In accordance with these results, pairwise between-population  $\Phi_{ST}$  showed a mixed pattern of differentiation between populations, which was slightly consistent with isolation of the eastern populations (particularly of the Swedish one, which was significantly different from four western continental populations and both island populations), but not with variation in migratory behavior or differences among biogeographic areas (Table 4). One sedentary population on the continent, Gibraltar, differed significantly from all other populations except those in the UK and on the Canary Islands (Table 4).

TABLE 1. Sequence differences among the 40 haplotypes found in blackcaps. Dots indicate identical bases, and numbers indicate insertions (the following bases inserted after the corresponding variable site: 1: A, 2: AA, 3: T, 4: C). The sequence of haplotype 1 has been deposited in GenBank with accession number AY500829.

Haplotype	Variable sites																														
	1	1	6	6	6	6	7	7	7	7	8	8	9	9	1	1	1	2	2	2	2	2	3	3	3	3	3	3	4	5	
1	T	G	A	T	G	A	G	G	T	T	C	G	C	A	G	G	T	A	A	T	C	A	T	T	T	A	T	C	G	T	A
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
5	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	1	.	.	.	.	.	A	.	.	A	.	.	.	.	A	.	G	.	.	.	.	.	.	G	1	A	A	G	.	.	
11	.	.	1	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	.	.	2	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	4	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
16	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G
21	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
22	.	.	1	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
23	.	.	.	.	.	.	.	3	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
25	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
27	3	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
28	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
31	.	.	.	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
34	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
36	.	A	1	3	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
37	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.
39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.
40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.

The small but significant differentiation between western and eastern populations of blackcaps was discernible in the neighbor-joining tree of haplotypes (Fig. 3). The phylogenetic relationships between haplotypes could not be resolved (the branch separating haplotypes 35 and 40 from the others was the only one supported by a bootstrap analysis, with a relatively low value of 51). Eastern populations were characterized by a higher frequency of haplotypes 2, 4, and their close relatives, whereas the group of haplotypes including number 1 was nearly exclusive of western populations (note that these groups are based on sequence differences, but differentiation between haplotypes is not large enough to define well supported clades in the tree). Blackcaps from Gibraltar were scattered in the tree, revealing a high haplotype richness (Fig. 3). The differentiation between this and other populations seemed to be mainly due to the high frequency of the haplotype 2 in this population, which also explains the com-

paratively low genetic diversity of this population (Fig. 2) despite harboring many haplotypes (Table 2). In fact, the U.K. and the Canary populations (not significantly different from Gibraltar) were the only other populations with a higher frequency of haplotype 2 than 1 (Table 2).

*Historical Population-Size Changes*

The analysis of mismatch distributions showed a significantly better fit to a model of changing population size than to one of constant population size in all populations except Gibraltar (Table 5). Thus, although Gibraltar approached a better fit to the sudden expansion model ( $P = 0.051$ ; Table 5), it was the only population in which the distribution of pairwise differences was not bell-shaped, as expected for a recently expanded population (Fig. 4). According to our estimates of  $\theta_0$ ,  $\theta_1$ , and  $\tau$ , and assuming an intermediate rate

TABLE 2. Distribution of haplotypes among blackcap populations. The number of private haplotypes in each population is also shown. The haplotypes distinguished by indels, not differentiated in the analyses from their homologues without alignment gaps, are labeled with asterisks. Haplotype 10 was excluded from all analyses. Populations are labeled as in Figure 1.

Haplotype	Western populations								Eastern		Atlantic		Total
	UK	Fra	Bel	Ger	NSp	CSp	Por	Gib	Swe	Aus	Mad	Can	
1	3	4	6	7	9	10	10	1	3	4	6	6	69
2	7	4	1	3	8	5	3	16	2	3	6	8	66
3	—	—	—	—	1	—	—	—	—	—	—	—	1
4	1	4	3	4	7	2	7	1	4	9	—	2	44
5*	—	—	—	—	1	—	—	—	—	—	—	—	1
6	—	—	—	—	—	1	—	—	—	—	—	—	1
7	—	—	—	—	—	2	—	—	—	—	—	—	2
8	—	—	—	—	—	1	—	1	—	—	—	—	2
9	—	—	1	—	—	1	—	—	—	—	—	—	2
10*	—	—	—	—	—	1	—	—	—	—	—	—	1
11*	—	1	1	—	—	—	—	1	—	—	—	—	3
12*	—	—	—	—	—	—	—	1	—	—	—	—	1
13*	—	—	—	—	—	—	—	1	—	—	—	—	1
14	—	—	—	—	—	—	—	4	—	—	—	—	4
15	—	—	—	—	—	—	—	—	1	—	—	—	1
16	—	—	—	—	—	—	—	—	1	1	—	—	2
17	—	—	—	—	—	1	—	—	2	—	—	—	3
18	1	—	1	—	—	1	—	—	—	—	—	—	3
19	—	—	—	—	—	—	—	—	1	—	—	—	1
20	—	—	—	—	—	—	—	—	—	2	—	—	2
21*	—	—	—	—	—	—	—	—	—	2	—	—	2
22*	—	—	—	—	—	2	—	—	—	—	—	—	2
23*	—	—	—	1	—	—	—	—	—	—	—	—	1
24	—	—	—	1	—	—	1	—	—	—	—	—	2
25	1	2	1	—	—	—	2	—	—	—	—	—	6
26	1	—	—	—	—	—	—	—	—	—	—	—	1
27	1	—	—	—	—	—	—	—	—	—	—	—	1
28*	—	—	—	—	—	—	—	—	—	—	1	—	1
29	—	—	1	—	—	—	—	—	—	—	—	—	1
30	—	—	1	—	—	—	—	—	—	—	—	—	1
31	—	1	—	—	—	—	—	—	—	—	—	—	1
32	—	1	—	—	—	—	—	—	—	—	—	—	1
33	—	—	—	—	—	—	1	—	—	—	—	—	1
34	—	—	—	—	—	—	1	—	—	—	—	—	1
35	—	—	—	—	—	—	—	1	—	—	—	—	1
36	—	—	—	—	—	1	—	1	—	—	—	1	3
37	—	—	—	—	—	—	—	—	—	—	1	—	1
38	—	1	—	—	—	—	—	—	—	—	—	—	1
39	—	—	—	—	—	—	—	—	—	—	—	2	2
40	—	—	—	—	—	—	—	—	—	—	—	1	1
<i>n</i>	15	18	16	16	26	28	25	28	14	21	14	20	241
Haplotypes	7	8	9	5	5	12	7	10	7	6	4	6	40
Private	2	3	2	1	2	4	2	4	2	2	2	2	

of evolution ( $\mu$ ) for the control region of 0.2 s/s/Myr, blackcaps would have expanded from a very small population size to an effective size of 9000 females around 7000 years ago. This estimated age of expansion is conditioned by the high uncertainty existing on the actual mutation rate of avian control regions. Thus, assuming a lower mutation rate (0.1 s/s/Myr) would put the expansion time back to 13,000 years ago, whereas considering a faster divergence (0.3 s/s/Myr) would move it forward to just 4000 years ago (Table 5).

#### *Between-Population Isolation and Migration*

Our nonequilibrium coalescent estimates of divergence time and migration rate supported previous results regarding population history (see estimates of scaled parameters for all pairwise between-population comparisons in Table 6). Pairwise comparisons between western continental populations,

eastern continental populations, and island populations showed that populations located to the east of the migratory divide diverged from western continental and island populations much before the latter diverged from each other (Fig. 5). This analysis also revealed restricted gene flow between populations located at either side of the migratory divide, and between continental (particularly eastern) and island populations, whereas migration rate was much higher between populations within all these geographic areas (Fig. 5).

Our estimates of divergence time and migration rate also supported the restricted gene flow between Gibraltar and other populations. The high  $\Phi_{ST}$  values obtained in comparisons between Gibraltar and other populations (see above) were not due to an earlier divergence: on average, Gibraltar diverged from other populations around 1900–5800 years ago (range corresponding to mutation rates for the control region be-



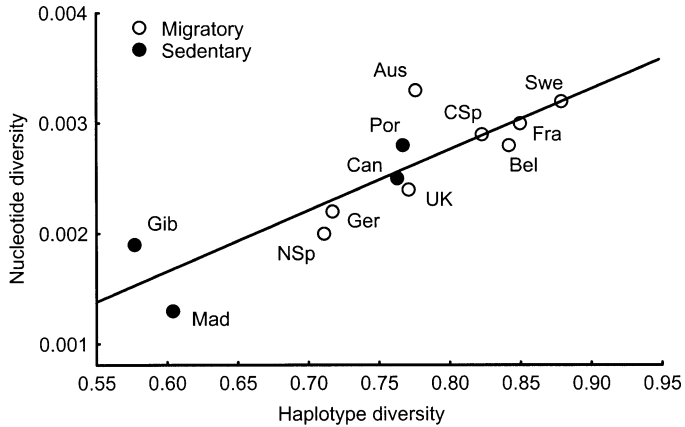


FIG. 2. Relationship between haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversity in 12 populations of blackcaps ( $r^2 = 0.75$ ,  $P < 0.001$ ). Populations are labeled as in Fig. 1.

tween 0.1 and 0.3 s/s/Myr), which is similar to divergence times obtained in comparisons between pairs of populations other than Gibraltar (1600–4800 years). However, the average migration rate between Gibraltar and other populations was five times lower than the rates obtained when comparing pairs of populations other than Gibraltar ( $m = 0.0002$ – $0.0006$  and  $m = 0.001$ – $0.003$ , respectively, assuming mutation rates between 0.1 and 0.3 s/s/Myr), even though the latter sometimes involved isolated populations from continental Europe and Atlantic archipelagos.

#### DISCUSSION

The diversity of migratory patterns of blackcaps spans the whole range from complete residence to long-distance migration toward tropical Africa, and includes short-distance migration between Mediterranean highlands and lowlands. Despite this large adaptive divergence among blackcap populations, only 3.6% of their molecular variance at the mitochondrial control region was due to between-population

differences, and only 0.15% of the variation, which was not significant statistically, was attributed to differences between migratory and sedentary populations. The unresolved phylogenetic relationships of haplotypes and the lack of population specific haplotype clades, together with coalescent estimates of divergence time and migration rate, indicate that any isolation between contemporary blackcap populations in continental Europe and the Atlantic islands, is of very recent origin. These observations rule out the idea of an ancient separation of migratory and sedentary lineages, and suggest that independent evolution of migratory behaviors has resulted in the extant complexity of migratory strategies exhibited by blackcaps. Our analyses of mismatch distributions and between-population divergence support a recent origin of this diversity, revealing a sudden expansion of blackcap populations from a bottlenecked population 4000 to 13,000 years ago, and a very recent effective between-population divergence. The fact that nonequilibrium estimates of divergence time were smaller than equilibrium estimates of time since population expansion (cf. Fig. 5 and Table 5) may be partially explained because recently diverged blackcap populations share a substantial amount of ancestral polymorphism. But discrepancy between divergence and expansion times (on average 4500 years) also leads to the interpretation that populations diverged some time after expansion had started, which is expected for a postglacial colonization process. Although the reliability of these estimates depends on the validity of our assumption of mutation rates for the blackcap's control region, our results are conservative in that they allow a wide uncertainty but still are consistent with palaeoecological data, supporting an expansion and divergence of blackcap populations coinciding with the retreat of ice sheets after the last glaciation (Hewitt 2000).

#### Postglacial Expansion and Evolution of Migration

The demographic trends experienced by blackcap populations revealed in this study are consistent with recent Pa-

TABLE 3. Results of AMOVA testing for among population differences and population structure in relation to migratory behavior, biogeographic area, and side of the migratory divide in central Europe. The analyses partitions total molecular variance into different components, whose significance was obtained by randomization after 5000 permutations.

Population structure tested	df	SS	Var. Comp.	% Var.	<i>P</i>
<b>No grouping</b>					
Among populations	11	15.18	0.029	3.57	0.010
Within populations	228	181.26	0.795	96.43	
<b>Migratory/Sedentary</b>					
Between groups	1	1.57	0.001	0.15	0.36
Among populations	10	13.61	0.029	3.49	0.016
Within populations	228	181.26	0.795	96.36	
<b>Continental/Iberian/Atlantic</b>					
Among groups	2	2.66	0.002	0.26	0.38
Among populations	9	10.69	0.024	3.17	0.016
Within populations	228	163.85	0.719	96.57	
<b>Western/Eastern</b>					
Between groups	1	2.74	0.030	3.90	0.021
Among populations	8	8.06	0.013	1.71	0.076
Within populations	196	143.69	0.733	94.39	



TABLE 4. Pairwise between-population differentiation in blackcaps. Below the diagonal, pairwise  $\Phi_{ST}$  (based on Kimura two-parameter distances between haplotypes under a gamma distribution with  $\alpha = 0.02$ ). Significant  $\Phi_{ST}$  are in bold. Above the diagonal,  $P$ -values for significant  $\Phi_{ST}$  obtained after 5000 permutations (\* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). Populations are labeled as in Figure 1.

	Western populations							Eastern		Islands		
	UK	Fra	Bel	Ger	NSp	CSp	Por	Gib	Swe	Aus	Mad	Can
UK	—											
Fra	-0.040	—						*				
Bel	0.017	0.006	—					***	*			
Ger	-0.037	-0.034	-0.032	—				*				
NSp	-0.041	-0.030	0.021	-0.031	—			*				
CSp	-0.034	-0.013	-0.006	-0.038	-0.028	—		*	*			
Por	-0.011	-0.011	-0.040	-0.034	0.002	-0.005	—	**	*			
Gib	0.050	<b>0.075</b>	<b>0.214</b>	<b>0.138</b>	<b>0.079</b>	<b>0.082</b>	<b>0.171</b>	—	*	*	***	
Swe	0.054	0.031	<b>0.164</b>	0.075	0.081	<b>0.101</b>	<b>0.118</b>	<b>0.100</b>	—		***	*
Aus	-0.017	-0.023	0.024	-0.028	-0.005	0.005	0.008	<b>0.091</b>	-0.007	—	*	
Mad	0.055	0.078	<0.001	0.052	0.083	0.013	0.016	<b>0.262</b>	<b>0.299</b>	<b>0.116</b>	—	
Can	-0.033	-0.005	0.060	0.007	-0.017	-0.010	0.043	0.029	<b>0.087</b>	0.016	0.083	—

learctic history. During Pleistocene glacial episodes, arborescent vegetation virtually disappeared from the Palearctic north of the main mountain ranges (Pyrenees, Alps, and Carpathians), causing a contraction of the range of the forest biota to a few mild areas around the Mediterranean basin. With the withdrawal of ice sheets after the last glacial maximum (18,000 years ago), forests expanded from Mediterranean refugia toward northern and highland areas (Hewitt 2000; Tzedakis et al. 2002), which allowed forest birds to expand their breeding ranges across the Palearctic (Hewitt 1996; Blondel and Mourer-Chauviré 1998; Taberlet et al. 1998). Newly colonized areas, located at higher latitudes or elevations and characterized by a seasonal cycle of produc-

tivity, imposed a selective force favoring the evolution of migratory behavior (Alerstam and Enckell 1979; Safrieli 1995).

The fact that several sedentary populations also show evidence of historical bottlenecks followed by sudden expansions, together with their nonsignificant differentiation from migrant populations (based on  $\Phi_{ST}$  estimates), and their relatively recent divergence times, suggest that blackcaps may have lost migratory behavior after a secondary colonization of mild areas. If sedentary blackcaps were remnants of populations occupying southern refugia during glacial periods, we should expect them to better fit a model of long-term constant population size, or at least to be somehow genetically distinct due to stochastic effects during independent history (Buerkle 1999). The loss of migratory behavior could have occurred on the Portuguese Coast, and it is the most likely explanation for the genetic characteristics of blackcaps from the Atlantic islands. Our analyses of genetic structure, isolation-by-distance effects, and  $\Phi_{ST}$  estimates support the hypothesis of the Atlantic islands being colonized by western migrant continental populations after the last glaciation, which entailed evolution of residency. Loss of migration after colonization of the Atlantic islands seems to have occurred in other passerine birds (Marshall and Baker 1999). This interpretation is further supported by our nonequilibrium coalescent estimates of divergence time, which reveal a recent divergence between populations from western continental Europe and the Atlantic archipelagos.

In contrast to other populations, sedentary blackcaps from Gibraltar did not show evidence of population expansion following the last glaciation. This is consistent with the fact that environmental conditions varied little in the southern Mediterranean during the ice ages, which appoints this area as a putative Pleistocene refuge for west-European biota (Hooghiemstra et al. 1992; Tzedakis et al. 2002). In particular, the forests of Gibraltar show many vegetation remnants from the Tertiary, indicating that the conditions found today are similar to those during the last episodes of glaciation (Hooghiemstra et al. 1992; Rivas-Martínez et al. 1997). In turn, blackcap populations could have remained sedentary in these habitats since long before the last glaciation. This possibility, along with the haplotype richness found in this pop-

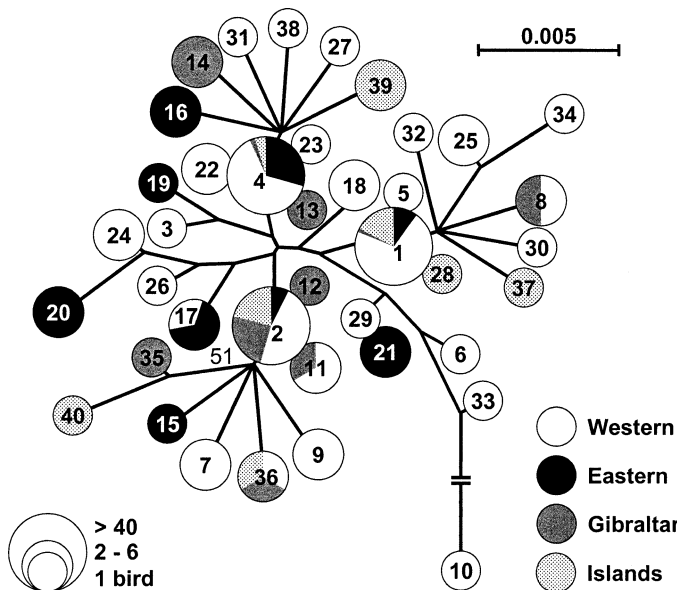


FIG. 3. Unrooted neighbor-joining phylogram of 40 blackcap mtDNA haplotypes. The size of the circles refers to the number of individuals (see Table 2 for details), and the shading identifies different population groups relevant for interpreting genetic structure. Haplotypes characterized by indels were manually placed on the tree, sitting on their homologues. The haplotype 10 was excluded from the analyses. Only one node received statistical support (the number above the branch indicates the percentage of 5000 bootstrap replicates in which the node was present).

TABLE 5. Parameters of the sudden expansion model (the chi-square tests examine its gain in fit compared to the constant size model; significant results,  $\alpha$  set at 0.05, indicate better fit to the expansion model), and estimates of age of expansion and female effective population size ( $N$ ) for the blackcap populations studied (labeled as in Figure 1). The expansion model assumes that an initial population at equilibrium with  $\theta = \theta_0$  (where  $\theta = 2uN$  is the expected pairwise difference among two randomly selected haplotypes, and  $u$  is the number of mutations per sequence per generation) grew to a new size at which  $\theta = \theta_1$ , and that this happened  $\tau$  units of mutational time before present. An average estimate obtained using an intermediate evolution rate for the control region (0.2 s/s/Myr), and the range resulting from mutation rates between the maximum in comparative studies (0.1 s/s/Myr; Baker and Marshal 1997) and the value obtained from human pedigrees (0.3 s/s/Myr; Sigurðardóttir et al. 2000) are shown. Populations are labeled as in Figure 1.

	Statistics of expansion					Years since expansion		Female $N$	
	$\tau$	$\theta_0$	$\theta_1$	$\chi^2(i)$	$P$	Average	Range	Average	Range
UK	1.219	0	3.38	5.75	0.016	6000	4000–12,000	8000	6000–17,000
Fra	1.516	0	5.65	7.79	0.005	8000	5000–15,000	14,000	9000–28,000
Bel	1.400	0	5.32	8.30	0.004	7000	5000–14,000	13,000	9000–26,000
Ger	1.117	0	2.53	5.74	0.017	6000	4000–11,000	6000	4000–13,000
NSp	0.978	0	2.46	9.72	0.002	5000	3000–10,000	6000	4000–12,000
CSp	1.459	0	4.66	13.16	<0.001	7000	5000–15,000	12,000	8000–23,000
Por	1.393	0	3.29	10.21	0.001	7000	5000–14,000	8000	5000–16,000
Gib	0.955	0	1.36	3.79	0.051	5000	3000–9000	3000	2000–7000
Swe	1.582	0	7.27	8.28	0.004	8000	5000–16,000	18,000	12,000–36,000
Aus	1.538	0.12	3.47	4.93	0.026	8000	5000–15,000	9000	6000–17,000
Mad	0.670	0	1.53	4.52	0.033	3000	2000–7000	4000	3000–8000
Can	1.268	0	3.22	6.27	0.012	6000	4000–13,000	8000	5000–16,000
All birds	1.312	0	3.73	89.65	<0.001	7000	4000–13,000	9000	6000–19,000

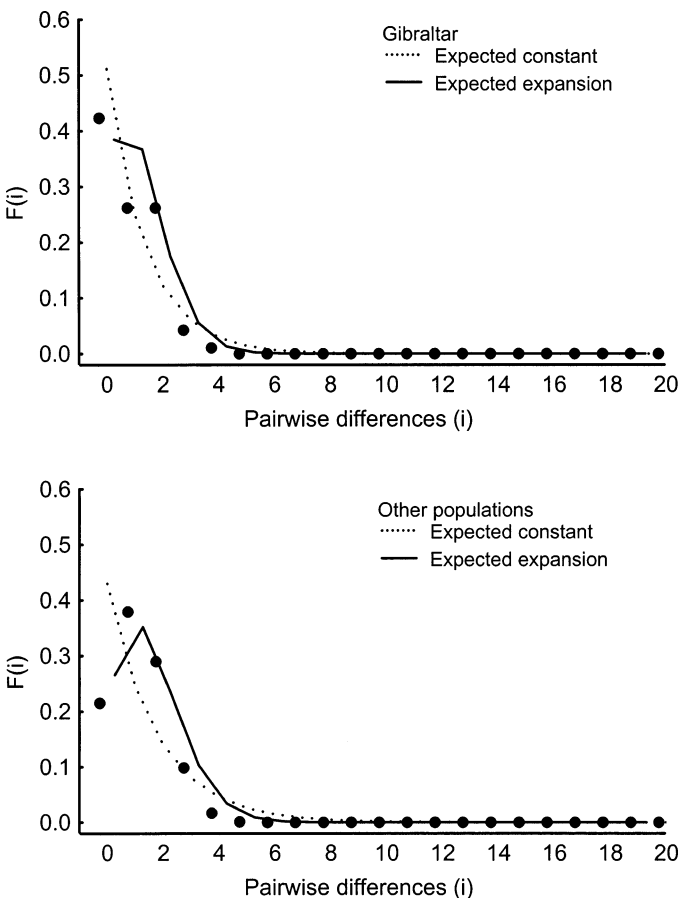


FIG. 4. Frequency distribution of pairwise nucleotide differences between haplotypes in Gibraltar and in other populations. Dots represent the observed frequencies, dashed lines indicate the expected frequencies under a constant population size model, and solid lines the expected frequencies under a sudden expansion model.

ulation (including a good representation of all major haplotype groups in the western part of the species' range), supports Gibraltar as a possible remnant of the ancestral populations from which western European blackcaps—and eventually Atlantic populations as well—expanded after the last glaciation.

#### Genetic Consequences of a Migratory Divide

Our analysis of genetic structure, isolation-by-distance effects,  $\Phi_{ST}$  estimates, and nonequilibrium coalescent estimates of divergence time and migration rate, support an earlier divergence and a limited gene flow between populations located at either side of the migratory divide in central Europe, compared to other groups of populations. This result supports the idea that blackcaps colonized eastern Europe from southeastern Pleistocene refugia (Hewitt 1996, 2000), and that migratory directions were constrained by colonization routes as populations would tend to winter in their original breeding grounds (Safriel 1995; Rugg and Smith 2002). The migratory divide, a narrow belt separating populations with different migratory behavior, would have been created by the secondary contact between populations colonizing Europe along western and eastern routes (Hewitt 1996). This circumstance might be common to many other European migrants, but its genetic footprint varies considerably between species. For example, great reed warblers *Acrocephalus arundinaceus* show a sharp genetic differentiation longitudinally across Europe, to the point that distinctive western and eastern clades of haplotypes can be identified (Bensch and Hasselquist 1999). Willow warblers *Phylloscopus trochilus* show clear differentiation in migratory behavior, and they even show stronger differences in morphology than great reed warblers, but there is no evidence of genetic differentiation in neutral markers between populations located at either side of the divide (Bensch et al. 1999). Blackcaps are intermediate between these two extremes, with haplotype frequencies dif-

TABLE 6. Maximum-likelihood estimates (scaled by effective population size,  $N$ ) of divergence time ( $t$ ) and migration rate ( $m$ ) between pairs of populations of blackcaps (Nielsen and Wakeley 2001). Values above the diagonal are estimates of  $T (= t/2N)$ , and those below the diagonal are estimates of  $M (= 2Nm)$ . Populations are labeled as in Figure 1.

	UK	Fra	Bel	Ger	NSp	CSp	Por	Gib	Swe	Aus	Mad	Can
UK		0.02	0.96	0.06	0.02	0.02	0.06	0.34	0.08	0.18	0.36	0.02
Fra	18.24		0.02	0.04	0.08	0.16	0.06	0.30	0.02	0.20	0.20	0.02
Bel	12.00	23.16		0.02	0.18	0.02	0.32	0.28	0.12	2.43	0.06	0.04
Ger	3.12	11.64	15.78		0.02	0.02	0.06	0.30	0.02	0.06	0.06	0.04
NSp	16.80	29.58	7.68	26.85		0.06	0.02	0.14	0.06	0.08	0.24	0.76
CSp	25.32	5.70	29.70	23.10	28.19		0.06	0.04	0.02	0.04	0.18	0.18
Por	11.58	28.32	27.18	21.05	6.32	13.92		0.34	0.14	0.18	0.14	0.04
Gib	6.12	1.38	1.14	1.26	2.44	3.42	0.99		0.26	0.12	0.20	0.06
Swe	3.24	26.52	6.66	6.60	2.84	28.88	2.38	1.51		0.14	0.46	0.08
Aus	2.16	2.82	27.24	3.48	2.73	4.18	7.71	1.04	20.04		0.40	0.24
Mad	6.90	1.20	7.86	2.96	1.86	9.11	1.54	0.99	0.75	0.81		0.20
Can	22.80	5.58	27.06	6.44	11.66	20.53	4.58	8.53	5.68	1.97	26.45	

fering significantly between western and eastern populations. This low differentiation could be due to a high historical gene flow between populations on both sides of the divide, and stochastic changes in haplotype frequencies during periods of isolation in Pleistocene refugia. Gene flow at the

contact zone is supported by the paradoxically high haplotype and nucleotide diversity of the Swedish population (Fig. 2), for which low values of both parameters would be expected according to stochastic lineage sorting during a leading-edge colonization advancing northwards (Hewitt 1996). However, this population is located near the contact zone with western populations (Fig. 1), and gene flow from the other side of the divide could enhance its genetic diversity.

At the continental scale, the genetic structure of European blackcaps and coalescent estimates of migration rate support the view that gene flow is restricted across the migratory divide, possibly due to a reduced fitness of hybrids between birds with very different migratory behaviors (Bensch et al. 1999). In the blackcap, hybrids between individuals with different migratory behavior show an intermediate migration pattern between the two parental phenotypes, which most likely confers a fitness disadvantage (Helbig 1991, 1996). However, blackcaps have sometimes overcome these restrictions to the evolution of migratory patterns, as shown by the rapid emergence of a new migratory direction (to northwest) in central European populations, which would otherwise migrate southwest (Berthold et al. 1992). This shows the great adaptability of migratory behavior in blackcaps.

#### Gene Flow and Diversity of Migratory Behaviors

Sedentary blackcaps from Portugal or the Atlantic archipelagos were not genetically different from western migratory populations (although our estimates of migration rate supported a recent restriction in gene flow between the continent and the Atlantic islands), showing that isolation between migratory and sedentary populations is not necessary for ecological differentiation. Differences in migratory behavior may be maintained by divergent selection due to different seasonality regimes, even though sedentary and migratory populations may maintain substantial gene flow. However, the sedentary population in Gibraltar seems to be unique within the blackcap's range. Our results show that this population is genetically differentiated from most other populations based on  $\Phi_{ST}$  estimates, it shows evidence of little, if any, expansion during historical times, and it maintains low gene flow with other populations. Other studies have shown that this population is adapted to Mediterranean

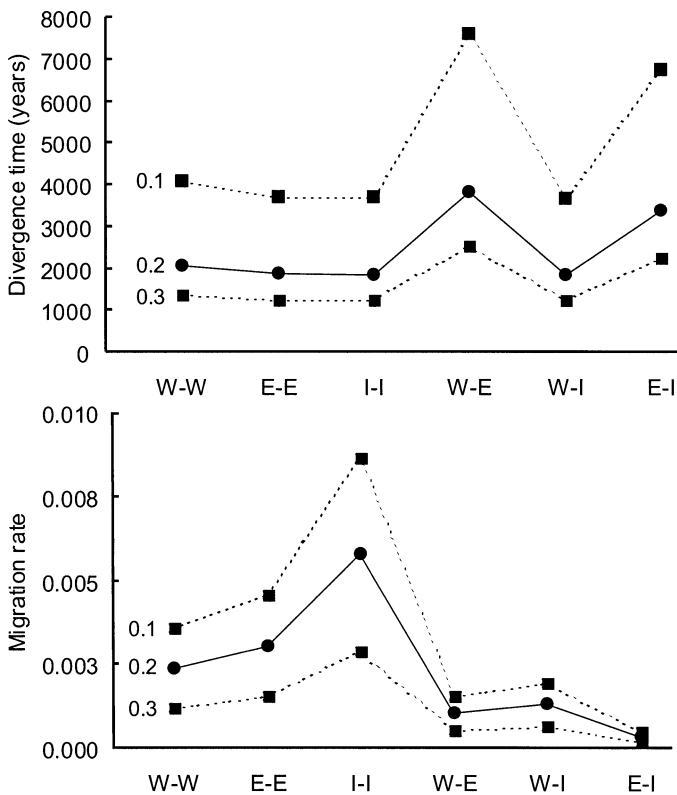


FIG. 5. Average coalescent estimates of divergence time and migration rate (proportion of migrants per generation) between pairs of populations of blackcaps (raw data are shown in Table 6). Pairwise comparisons have been grouped according to geographic areas (W, western continental populations; E, eastern continental populations; I, island populations). Different plots are shown for an average evolution rate for the control region (0.2 s/s/Myr), and the range resulting from mutation rates between the maximum in comparative studies (0.1 s/s/Myr; Baker and Marshal 1997) and the value obtained from human pedigrees (0.3 s/s/Myr; Sigurðardóttir et al. 2000).

forests, as indicated both by morphological traits (Tellería and Carbonell 1999) and a better reproductive output than Iberian migrants (Gibraltar juveniles show greater developmental stability and better body condition than juveniles of migrant individuals) despite the lower productivity of Mediterranean habitats (Carbonell et al. 2003). Such differences are manifested in sympatry during winter, when large-sized sedentary blackcaps outcompete arriving migrants forcing them to occupy less favorable habitats (Pérez-Tris and Tellería 2002b). Other studies have found significant ecological and genetic differences between sedentary and migratory bird populations, supporting the existence of cryptic population boundaries in the range of migratory species that should be taken into account in conservation planning (Buerkle 1999).

#### *Population History and the Future of Migratory Birds*

The potential of sedentary populations to rapidly acquire migratory behavior has been documented both in laboratory and in field studies (Berthold 1996; Able and Belthoff 1998). Remarkably, some of the most illuminating experiments have been done in blackcaps (Helbig 1996; Pulido et al. 1996, 2001; Pulido and Berthold 2003), and this species has also provided one of the most impressive examples of rapid evolution of new migratory patterns in natural populations (Berthold et al. 1992). The question remains as to what extent rapid changes in migratory pattern may be paralleled by morphological, physiological, and life-history adaptations forming highly divergent migratory strategies like the ones exhibited by blackcaps across their range. Given the strong selection forces related to migratory behavior and the high heritability of traits such as timing, orientation, and distance of migration (Berthold 1996; Pulido et al. 2001), morphological traits associated with migration (Fiedler 1998), or fat deposition dynamics (Berthold 1996), rapid adaptive evolution of complex combinations of attributes forming migratory strategies seems feasible. In fact, considerable changes in morphology, behavior, or physiology may take place in just a few generations, as shown in experimental studies (Losos et al. 1997; Orr and Smith 1998). In natural populations, this process is revealed by intraspecific variation in morphology, behavior, or life history without divergence at rapidly evolving neutral genetic markers (Nagel and Schluter 1998; this study).

The facility for birds to acquire or lose migratory behavior may depend on factors other than the response of migration to selection in changing environments. For example, some European partially migratory birds introduced in New Zealand lost migratory behavior straight away (Veltman et al. 1996), showing that environmental influences might speed up changes in migration patterns. However, phenotypic plasticity is unlikely to explain the morphological and physiological adaptations associated to migratory behavior, therefore acquisition of migration is more likely to be the outcome of selection in seasonal habitats. On the other hand, rapid evolution of migratory behavior might be limited by the likely slower response of other, less mobile organisms migratory birds rely upon (for example, the plants they use for breeding or feeding). In fact, the postglacial expansion of European

forests apparently set the tempo of evolution of migratory strategies in blackcaps.

In summary, our results show that both migration and residency have evolved independently on several occasions in the recent history of blackcaps, generating an impressive diversity of migratory strategies. This has important evolutionary implications, especially because of ongoing global climate change (Both and Visser 2001). According to our results and other studies (Berthold et al. 1992; Able and Belthoff 1998; Pulido et al. 2001), birds would be able to respond to global warming by changing migratory behavior, adjusting almost immediately—evolutionarily speaking—their migratory distances and heading directions, and developing in the long run a complex array of physiological, morphological, and ecological adaptations. However, our results also stress that the persistence of sedentary populations in glacial refugia is probably necessary as a source for colonization of seasonal areas during successive glacial waves (Hewitt 1996, 2000). This means that the preservation of the usually small and geographically localized sedentary populations is crucial for the long-term conservation of temperate migratory birds (Taberlet and Cheddadi 2002), and shows that looking at the past may help us to understand current evolutionary processes and to improve future strategies to preserve biodiversity.

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