

Mitochondrial DNA sequence variation and phylogeographical structure of rock partridge (*Alectoris graeca*) populations

VITTORIO LUCCHINI & ETTORE RANDI*

Istituto Nazionale per la Fauna Selvatica, 40064 Ozzano dell'Emilia (BO), Italy

The rock partridge (*Alectoris graeca*) is distributed in the Alps, Apennines and Balkans, in mountainous areas that were heavily affected by cyclic climate and landscape changes during the last Pleistocene glaciations. Some partridge populations have colonized and expanded their present ranges only after deglaciation and recent deforestation by humans. Other populations have been segregated in land-bridge islands isolated after the rise in level of the Mediterranean Sea. Consequently, partridges from different areas could be genetically differentiated. This study has analysed 436 nucleotides of the domain I of the mitochondrial DNA control-region in 70 rock partridges collected from 14 populations throughout their distribution. Phylogenetic analysis and analysis of molecular variance showed that the Sicilian population is very divergent from the continental populations. The Albanian and central Apennine samples are slightly divergent from the Alpine rock partridges, which cluster in two groups according to their east–west distributions. The mitochondrial DNA sequences suggest the existence of a phylogeographical structuring among rock partridge populations, resulting from genetic divergence in southern refugia and subsequent postglacial colonization of northern mountain areas. Sicilian partridges could have been isolated for $\approx 500\,000$ years, whereas the Albanian–Apennine populations could have been in contact since the last glacial maximum (21 000 yr BP) through the north Adriatic land-bridge. The Alps could have been colonized in two different periods or by two different source populations. The mitochondrial DNA phylogeographical structuring is not completely concordant with the subspecies distribution. These findings suggest that rock partridges should be managed based on the identification of phylogeographical units.

Keywords: *Alectoris graeca*, conservation units, mtDNA control-region, phylogeography, post-glacial expansion, rock partridge.

Introduction

Climate and landscapes have been strongly affected by periodic glaciations during the last 2.5 million years in the Northern Hemisphere, and particularly during the late Pleistocene, when at least 10 glaciations occurred in a period of one million years, with a cold maximum approximately every 100 000 years (Roy *et al.*, 1996). The last glacial–interglacial stage (the Würm–Holocene of western Europe) was punctuated by repeated cyclical stadial and interstadial events, and abrupt climate changes have also been recorded in European, Asian and tropical regions (Stager & Mayewski, 1997; Thompson *et al.*, 1997). Towards the end of the Würmian glaciation at the

last glacial maximum (21 000–18 000 yr BP) ice sheets covered the Alps and the Pyrenees, and glaciers were also scattered across the Apennines and the Balkans. Enormous quantities of water were frozen in glaciers, the Mediterranean Sea basins were about 100–120 m lower than today and land-bridge islands were connected with the continent (Peltier, 1994; Fig. 1).

Quaternary landscape and climate changes had dramatic consequences on the distributions of European and Mediterranean plant and animal communities (Hewitt, 1996). Cold climates led to extinction of northern populations, which could have persisted in southern refuge areas. Comparative phylogeographical studies have recognized the existence of three potential refugia in southern Iberia, Italy and

*Correspondence. E-mail: met0217@iperbole.bologna.it

in the Balkans (Taberlet *et al.*, 1997). Definitive Holocene climate warming at the end of the Younger Dryas, about 10 000–7000 yr BP (Roberts, 1989; Stager & Mayewski, 1997), led to a northward expansion of populations from their glacial refugia, following peculiar and not strictly concordant post-glacial colonization routes (Taberlet *et al.*, 1997).

Mountain biota can provide models to describe patterns of postglacial colonization and to test for their genetic consequences on population diversification (Hewitt, 1993, 1996). The rock partridge (*Alectoris graeca*, Aves, Galliformes) is a polytypic species, with four described subspecies (Cramp & Simmons, 1980): *A. g. saxatilis* (Alps, north-western Balkans); *A. g. graeca* (south-western Balkans, Greece, south Bulgaria); *A. g. whitakeri* (Sicily); and *A. g. orlandoi*

(Italian Apennines; Priolo, 1984). It is patchily distributed, preferring dry, open rocky mountains, although it has a wide ecological tolerance, breeding from the sea level in the eastern Adriatic coast and Greece, up to 3000 m altitudes in the Italian Alps (Bernard-Laurent & de Franceschi, 1994). Rock partridges originated about 2.4 Ma (million years ago), approximately at the Pliocene/Pleistocene boundary (Randi, 1996). Their distributions would have fluctuated repeatedly during Pleistocene glacials and interglacials, and the present populations could not have colonized the Alps before 6–8000 yr BP ago (Blondel, 1986; Randi, 1996). Therefore, the rock partridge is an interesting case-study to investigate the consequences of Pleistocene climate and habitat changes on the geographical distribution of intraspecific genetic variability.

Mitochondrial DNA (mtDNA) sequencing has often revealed huge amounts of genetic variability within and among conspecific populations (Avise, 1994). Significant geographical mtDNA structuring might evolve in a relatively short time if physical or behavioural barriers significantly reduce dispersal and gene flow among populations, particularly in species with dispersal bias against females and with maternal phylopatry (Avise *et al.*, 1987). Intraspecific phylogeographical patterns can also result from the persisting effects of historical factors which have shaped the genetics of populations through demographic fragmentation and bottlenecks, extinctions and recolonization (Hewitt, 1993, 1996).

In this paper the hypervariable domain I of the mtDNA control-region (D-loop) is analysed in rock partridge populations sampled across their distributions. The aims of this study are: (i) to describe mtDNA sequence variation within and among local populations; and (ii) to reconstruct their phylogeographical relationships within the background of Pleistocene and recent habitat changes. Accurate reconstructions of intraspecific phylogeography are essential for understanding the biogeographical histories of single species and entire regions (Avise *et al.*, 1987; Taberlet *et al.*, 1997), as well as for implementing scientifically based conservation strategies effective in preserving genetic diversity within and among populations (Moritz, 1994).

Materials and methods

Sample collection, DNA extraction, amplification and sequencing

Total DNA was extracted using guanidinium thiocyanate (GuSCN) and diatomaceous silica particles

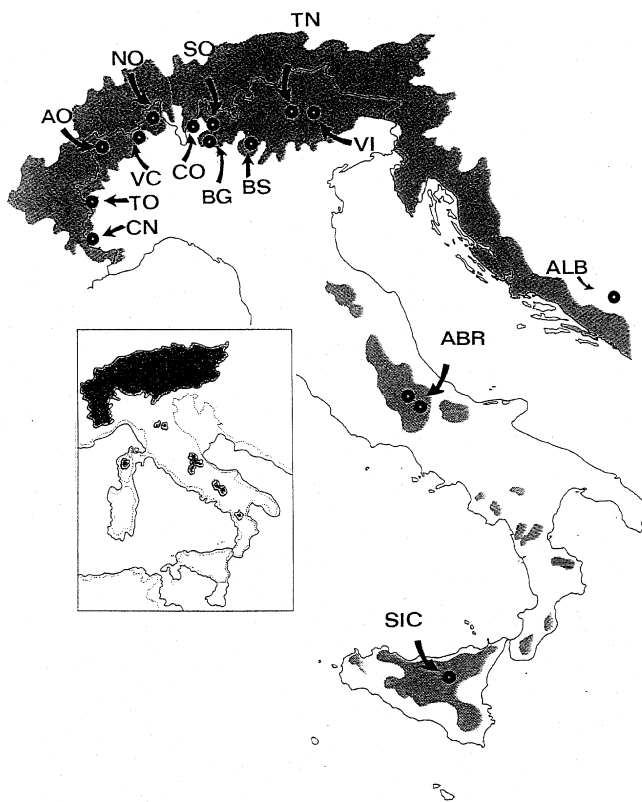


Fig. 1 Map showing the sample localities and approximate geographical range of the rock partridge in Italy and the north-western Balkans. The insert describes the hypothetical coastal lines of the Italian peninsula and glacier ranges (dark areas) at the last glacial maximum (21 000 yr BP). Acronyms of the sampled populations: SIC, Sicily; ABR, central Apennines; CN, Cuneo; TO, Torino; AO, Aosta; VC, Vercelli; NO, Novara; CO, Como; SO, Sondrio; BG, Bergamo; BS, Brescia; TN, Trento; VI, Vicenza; ALB, Albania.

(SIGMA D5384) (Gerloff *et al.*, 1995) from 95% ethanol-preserved liver tissues of samples collected from localities mapped in Fig. 1. The following outgroup sequences were obtained (locality, GeneBank accession number): (i) red-legged partridges: *A. rufa*-1 (Spain, AJ222740), *A. rufa*-2 and -3 (Portugal, AJ222739); (ii) chukar partridges: *A. chukar*-1 and -2 (Israel, AJ222728), *A. chukar*-3 (Kirghizistan, AJ222729).

The entire mtDNA D-loop was PCR-amplified using the external primers PHDL (tRNA^{Glu}; 5'-AGGACTACGGCTTGAAAAGC-3') and PHDH (tRNA^{Phe}; 5'-CATCTTGGCATCTTCAGTGCC-3') (Fumihito *et al.*, 1995). Sequences of the hypervariable domain I (Baker & Marshall, 1997; Randi & Lucchini, 1998) were obtained by double-stranded DNA cycle sequencing with the ABI Prism Dye Terminator kit in an ABI 373 automatic sequencer, using primer PHDL and the internal primer PH-H521 (5'-TTATGTGCTTGACCGAGGAAC-CAG-3'), which maps in the conserved central domain of the avian D-loop (for details on the laboratory protocols, see Randi & Lucchini, 1998).

Sequence analysis

Sequence variability, pairwise genetic distances and neighbour-joining trees (NJ) were computed using MEGA (Kumar *et al.*, 1993). Compositional stationarity and substitution models were tested using MODELS (Rzhetsky & Nei, 1995). Phylogenetic relationships among mtDNA haplotypes were obtained by maximum likelihood (ML) as implemented in PUZZLE 3.1 (Strimmer & von Haeseler, 1996) and by maximum parsimony (MP) with PAUP 3.1 (Swofford, 1993). Statistical support of topologies was assessed by bootstrap (Felsenstein, 1985), with 1000 resamplings followed by neighbour-joining (bootstrap confidence level BCL; MEGA) or parsimony (50% majority rule consensus tree; PAUP) reconstructions. Support for ML trees was evaluated by the reliability percentages (RP), e.g. the number of times the group appears after 1000 ML puzzling steps (PUZZLE). Heterogeneity of the distributions of mtDNA haplotypes among populations was evaluated by χ^2 analyses through 1000 Monte Carlo randomizations of the original data (Roff & Bentzen, 1989). A hierarchical analysis of molecular variance was performed using AMOVA (Excoffier *et al.*, 1992), with the number of pairwise nucleotide substitutions as input matrix of sequence divergence (L. Excoffier, pers. comm.). AMOVA estimates the haplotypic correlations (Φ -statistics) within and among different partitions of the data set, and takes

into account both haplotype frequencies and nucleotide divergence among haplotypes. Φ -statistics can be used as hierarchical F -statistics of geographical subdivisions (Cockerham, 1973), and Φ_{ST} values are analogous to F_{ST} and indicate the extent of geographical subdivisions. Significance of Φ -statistics was obtained by randomization. Haplotype [$H = (1 - \sum_i x_i^2)/(n-1)$] and nucleotide [$\pi = \sum_{ij} x_i x_j \pi_{ij}$] diversities, where x_i = population frequency of the i th haplotype; x_j = population frequency of the j th haplotype; and π_{ij} = proportion of nucleotide differences between i th and j th haplotypes, were computed following Nei (1987).

Results

Sequence variability

In 70 rock partridges and six chukar and red-legged partridge outgroups, 436 nucleotides of the mtDNA D-loop domain I have been sequenced. The 24 variable sites (5.5% of the entire sequence) defined 13 haplotypes, 11 distributed among the Alpine, Apennine and Albanian samples, and two among the Sicilian samples (Fig. 2). These haplotypes were distinct from each other by one to 17 substitutions. Seven haplotypes were defined by single autapomorphic substitutions (haplotypes nos 1, 2, 3, 4, 5, 10, 11). The 12 substitutions distributed among the Alpine, Apennine and Albanian samples were all transitions (Ti), whereas the two Sicilian haplotypes had one Ti and one transversion (Tv) difference. Continental and Sicilian haplotypes differed for a maximum of 17 substitutions, with four Tv (Fig. 2). The chukar and red-legged partridge outgroups had distinct D-loop haplotypes.

The bulk of sequence variability was distributed in a region encompassing nucleotides 200–400 (Fig. 2), and corresponding to the hypervariable part of D-loop domain I of galliforms (Randi & Lucchini, 1998). The average sequence divergence among rock partridge haplotypes was 1.5%, with values ranging from 0.23%, among Alpine haplotypes, to 3.7%, between haplotypes nos 13 (Sicily) and 1–5 (Alps). Divergence among rock partridges and the outgroups was 4.1% to 6.0%, e.g. it was less than two times the maximum intraspecific divergence among rock partridges. The average Ti : Tv ratio was undetermined within continental rock partridges because of the absence of Tv (Fig. 2), it was 3.56 between continental and Sicilian rock partridges, 3.81 between rock and chukar partridges, and 1.89 between rock and red-legged partridges. Average nucleotide composition of rock partridge sequences

[illegible]

Fig. 2 The observed variable positions in 436 nucleotide sequences of the mtDNA D-loop of partridges. Localities are mapped in Fig. 1. Numbers at the head of the columns refer to the control-region sequences of *Alectoris* (Randi & Lucchini, 1998). Transversions are in bold.

was: 28% A, 29% C, 29% T and 14% G, as usual for avian control-regions (Baker & Marshall, 1997; Randi & Lucchini, 1998). Nucleotide composition was stationary ($I = 14.83$; $P < 0.001$), and Kimura 2-parameters or Tamura–Nei models were appropriate estimators of genetic distances (Rzhetsky & Nei, 1995).

Phylogeographical structure

The Sicilian rock partridges had two unique mtDNA D-loop haplotypes (nos 12 and 13; Fig. 2), very divergent from all the other sampled sequences. The Albanian populations had three unique haplotypes (nos 9, 10 and 11; Fig. 2) and shared haplotype no. 7 with the Apennine partridges. The second and most frequent Apennine haplotype, no. 8, was shared with five Alpine populations distributed in the western and central parts of the range (Figs 1 and 2). The Alpine western populations (CN, TO and AO) had two haplotypes (nos 1 and 2), which were absent in the eastern populations (Figs 1 and 2). On the other side, the eastern populations (VI and TN) had two haplotypes (nos 5 and 6), which were widespread across the Alps, but did not have haplotype no. 8, distributed across the Apennines and the eastern

and central Alps (Figs 1 and 2). Only two haplotypes (nos 6 and 8) were shared by many different populations (Fig. 2).

Neighbour-joining trees (NJ) of rock-partridge D-loop sequences were computed using Tamura–Nei γ distances with α -values ranging between 0.05 and 0.5 (Fig. 3). The different α -values had no effects on this topology, but modified the relative lengths of long vs. short internal branches, which determined slightly different bootstrapping values for internodes (not shown). Using red-legged and chukar as the outgroups, rock partridge sequences were joined in a strongly supported monophyletic assemblage (BCL = 91%; RP = 100%; 50% majority rule = 77%). The widely divergent Sicilian rock partridges joined in cluster A supported by 100% bootstrap values. Albanian, Apennine and Alpine rock partridges grouped in a strongly supported cluster (BCL > 80% with $\alpha = 0.3$ –0.5; RP = 98%; 50% majority rule = 86%), which was subdivided into: (i) cluster B, including three of the four haplotypes found in Albanian partridges; (ii) cluster C, including haplotype no. 7, which was shared by one Albanian and six Apennine samples, all the Apennine partridges, and most of the haplotypes found in the western and central Alps; and (iii) cluster D, with

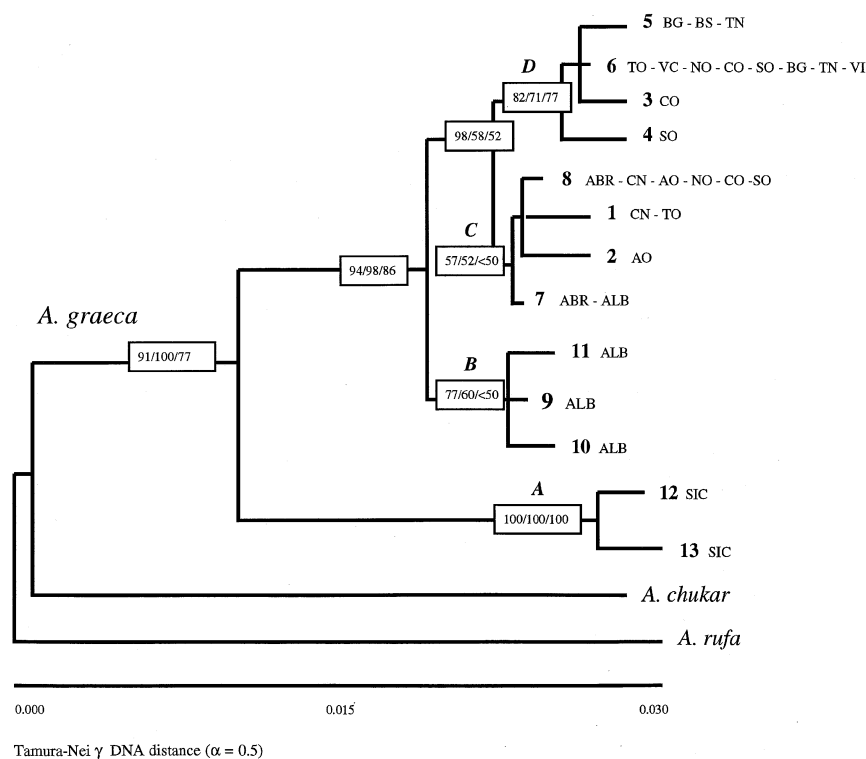


Fig. 3 Neighbour-joining tree based on Tamura-Nei γ distances ($\alpha = 0.5$) among mtDNA D-loop haplotypes of partridges. The haplotypes are numbered as in Fig. 2. Sampling localities are mapped in Fig. 1. Numbers at internodes indicate bootstrap confidence level, reliability percentages and 50% majority rule bootstrapping support, respectively.

the haplotypes distributed mainly in central and eastern Italian Alps.

Similar topologies were reconstructed by the ML method (PUZZLE), using the Tamura-Nei model with uniform or heterogeneous discrete γ -distributed rates (the RP values of the ML tree are reported in Fig. 3). The single topological difference between the NJ and ML trees was the position of haplotype no. 7, which shifted from a weak linkage to cluster C (in the NJ tree) to a weak linkage to cluster B (in the ML tree). Branch-and-bound MP searches produced a single tree (length $L = 16$, consistency index $CI = 0.89$, retention index $RI = 0.93$; unweighted parsimony excluding uninformative positions, with 10 replicates of random addition of terminal sequences, TBR branch-swapping and MUPARS option), with topology similar to NJ and ML trees, but with completely unresolved relations among the haplotypes within each of the four clusters as defined above.

Neighbour-joined, ML and MP trees indicate the existence of a phylogeographical structure among rock partridge populations. Phylogenetic relationships and geographical distributions of D-loop haplotypes are concordant: the primary subdivision is between Sicilian and continental rock partridges; then, continental rock partridges are subdivided into Albanian-Apennine vs. Alpine populations. The

Albanian and Apennine populations share identical or very similar haplotypes, whereas the Alpine populations include two D-loop lineages, which are distributed with different frequencies among western vs. central-eastern populations.

Significance of geographical heterogeneity

Phylogenetic relationships among the haplotypes (Fig. 3), and their distributions among the different localities suggest a subdivision of the total sample of 14 populations into three regional groups: (I) Sicily (one population, $n = 10$); (II) Apennines+Albania (two populations, $n = 19$); and (III) Alps (11 populations, $n = 41$). Haplotype frequencies were significantly different among the whole set of 14 populations ($\chi^2 = 307$; $P < 0.001$). The major contributions to geographical heterogeneity were given by the Sicilian, Albanian and Apennine populations. Accordingly, the χ^2 -values dropped down to 144 ($P < 0.001$), when Sicily was omitted, and to 89 ($P < 0.040$), when Sicily, Apennines and Albania were omitted from the comparisons.

The analysis of molecular variance (Table 1) indicated that 83.85% was distributed among the undivided 14 populations, with significant $\Phi_{ST} = 0.838$ ($P < 0.002$). The division of populations into three groups (as defined above) increased the value of Φ_{ST}

Table 1 Analysis of molecular variance in rock partridge populations. *A* = among-regions component of variance; *B* = among-population/within regions component of variance; *C* = within-population component of variance; *P* = probability of obtaining a more extreme random variance component. Φ -statistics are explained in the text

Groups	Divisions	% Variance	Φ -statistics	<i>P</i>
Global Φ_{ST}	Among 14 populations	<i>A</i> = 83.85	$\Phi_{ST} = 0.838$	<0.002
	Within populations	<i>C</i> = 16.15		
Three regions	Sicily	<i>A</i> = 79.58	$\Phi_{CT} = 0.796$	0.094
	Albania + Apennines	<i>B</i> = 8.98	$\Phi_{SC} = 0.440$	<0.002
	Alps	<i>C</i> = 11.44	$\Phi_{ST} = 0.886$	<0.002
13 populations	Excluding Sicily	<i>A</i> = 57.19	$\Phi_{ST} = 0.572$	<0.002
		<i>B</i> = 42.81		
11 populations	Excluding Sicily, Albania and Apennines	<i>A</i> = 35.43	$\Phi_{ST} = 0.354$	<0.002
		<i>B</i> = 64.57		

to 0.886 ($P < 0.002$). In this case, 79% of total variance was distributed among the three geographical groups, and 9–11% was distributed among populations/within groups and within populations, respectively. Haplotypic correlations among groups were not significant ($\Phi_{CT} = 0.796$; $P = 0.094$; Table 1). If the Sicilian group is omitted from the analyses, the values of variance and haplotypic correlation among groups drop to 57% ($\Phi_{ST} = 0.57$; $P < 0.002$). If the Sicilian, Albanian and Apennine groups are omitted, the values of variance and haplotypic correlation among groups drop to 35% ($\Phi_{ST} = 0.35$; $P < 0.002$), respectively, suggesting once again that the major contribution to geographical differentiation is attributable to the insular population.

Discussion

Rock partridge populations sampled across the Alps, central Apennines, Albania and Sicily are genetically structured. In particular, the Sicilian rock partridges have two unique mtDNA D-loop haplotypes, highly divergent from all the other sampled sequences. Phylogenetic trees indicate the existence of a major genetic gap between the Sicilian and the continental rock partridges (Fig. 3). The average genetic distance between the Sicilian and the other rock partridge populations is 3.7%, corresponding to the 60% of the average genetic distance among the three studied species of *Alectoris* (5.9%), and contrasting with the small average sequence divergence among continental rock partridge populations (0.9%). Albanian and Apennine partridges are closely related (average genetic distance is 0.7%). The haplotypes of the Alpine partridges join in clusters C and D, with an average genetic distance of 0.5% (Fig. 3). These clusters include haplotype no. 8, which is widespread in the Apennines and western

Alps, and haplotype no. 6, which is widespread across the Alps but is absent from the Apennines (Fig. 3). About 79% of the total molecular variance is distributed among the three main geographical areas (Sicily, Albania–Apennines, Alps). Estimates of genetic diversity within the three geographical groups are similar, with $H = 0.53$, 0.71, 0.23 and $\pi = 0.0011$, 0.0013, 0.0017 in Sicily, Albania–Apennines and Alps, respectively.

This phylogeographical pattern can be interpreted against the background of Pleistocene events. During the last major Pleistocene glaciations the Italian coastal lines were very different from present, and Sicily was connected to peninsular Italy (Fig. 1). At the Würmian glacial maximum the northern Adriatic basin was a dry alluvial plain with boreal forests in the north and temperate forests in the south. The Alps were completely glaciated, subalpine plains were cold tundra on loess, and there were glaciers also in the Apennines (Fig. 1). Steppe and sand deserts were widespread throughout most of the lowlands in southern Europe and central Asia. The main glacial refugia for the Mediterranean-type plant communities were in Spain, southern Italy, Sicily, southern Balkans and the northern border of the Caspian sea (Taberlet *et al.*, 1997).

Lowland and mountain open shrublands and steppes are the typical habitats of *Alectoris*, which probably evolved at the Pliocene/Pleistocene boundary and during Pleistocene glacials in southern European and Asian steppes, as well as in the isolated Mediterranean refuges of south-western Europe (Randi, 1996). Postglacial expansion of rock partridge populations, and of the other Mediterranean *Alectoris* as well, could have been fostered by the consequences of human impacts on vegetation. Deforestation in the Alps was intense between 6000 and 4500 years ago (Naveh & Vernet, 1991).

During that period the use of domesticated grazing animals and cultivation of cereals became widespread in Europe (Naveh & Vernet, 1991). The synergistic consequences of deforestation and agriculture probably produced a rapid extension of an ecological niche suitable for partridges.

Climatic and habitat conditions dictate that rock partridges were absent from the Alps, subalpine plains and northern Apennines during the Würmian glaciation. There are some differences in haplotype frequencies among western and eastern Alps: only the eastern and central Alpine rock partridges have haplotype no. 8, which is the most frequent one in the central Apennines, whereas central and eastern Alpine populations have some unique haplotypes with restricted distribution (nos 3, 4, 5). The Alps could have been colonized by partridges in different temporal waves or, alternatively, by two different source populations. One source population could have survived during the last glaciation in a north-eastern Balkan refuge and have colonized the Alps, starting from the eastern part towards the centre of the range. This population is represented by the haplotypes joined in cluster D (Fig. 3). Another source population could have survived in southern Apennine-Albanian refuges, and have colonized first the northern Apennines, then the western Alps, moving towards the centre of the range (cluster C in Fig. 3). An improved resolution of the phylogeography of the rock partridges needs larger sample collections from throughout the Balkan range. Partridge populations of the central Balkans, central Italian Apennines, southern Italy and Sicily, could have been in reciprocal contact through land connections and by suitable habitat corridors since the last glacial maximum (at 21 000 yr BP). However, the deep genetic divergence among Sicilian and continental partridges suggests that the insular population has been genetically isolated for a much longer time. In fact, Sicilian rock partridges bear phylogenetically basal mtDNA haplotypes that are not contemporaneous to the most derived peninsular and continental ones (Fig. 3). Sicily was repeatedly connected and isolated during Pleistocene glacials and interglacials in the last 2.5 Myr. Therefore, rock partridges could have colonized Sicily before the last glaciation and could have remained genetically isolated since then.

The Apennine and Alpine postglacial colonization routes originating from refuge areas in southern Italy and the southern Balkans have been described also in other species [i.e. the brown bear (*Ursus arctos*), the grasshopper (*Chorthippus parallelus*), the silver fir (*Abies alba*), the common beech (*Fagus*

sylvatica) and the white oaks (*Quercus* sp.)], and represent one of the main phylogeographical patterns produced by historical factors among western European animal and plant populations (as summarized and discussed by Taberlet *et al.*, 1997).

It is difficult to calibrate the substitution rates of mtDNA D-loop (Loewe & Scherer, 1997), as domains and nucleotide sites of the D-loop evolve at very heterogeneous rates. In particular, in the D-loop of *Alectoris* most sites are apparently invariant, and the few sites that are free to mutate are hypervariable (Randi & Lucchini, 1998). Hypervariable sites tend to saturate quickly, and substitution rates might also be grossly underestimated in comparisons among sequences that recently diverged. With these drawbacks in mind, information on interspecific divergence among sequences of the entire D-loop of red-legged, chukar and rock partridges (Randi & Lucchini, 1998) can be used to calibrate the intraspecific rate of D-loop change. Biochemical and molecular data (Randi, 1996) suggested the following phylogenetic relationships and divergence times among these species: (*A. rufa*: 2.5 Myr; *A. chukar*, *A. graeca*: 2.0 Myr). As Ti became quickly saturated, only the number of Tv are counted that have accumulated during interspecific divergence among these species, e.g. 21.5 and 18.5 Tv since divergence of *A. chukar* and *A. graeca* from *A. rufa*, respectively, and 11 Tv since divergence between *A. chukar* and *A. graeca*. The number of Tv per million years in the three lineages are: 8.6, 7.4 and 5.5, respectively. There are seven Tv differences between the entire D-loop sequences of Sicilian and peninsular rock partridges, corresponding to time $t = (7/8.6)/2 = 0.41$, $(7/7.4)/2 = 0.47$ and $(7/5.5)/2 = 0.64$ Myr of independent evolution of the Sicilian haplotypes. These calibrations place the origin of Sicilian rock partridge haplotypes at $\approx 500\,000$ years ago, roughly corresponding to the Günz-Mindel interglacial. However, these divergence times could be overestimated, because: (i) the rate of Tv saturation increases with interspecific divergence, and consequently Tv rates per million years will be underestimated; (ii) interspecific Tv rates are based on only two to three individual sequences and intraspecific polymorphisms are not taken into account; and (iii) retained ancestral haplotypes can predate the origin of Sicilian populations.

The observed mtDNA phylogeographical structuring is not completely concordant with the distribution of the described subspecies of rock partridge, which were recognized by slightly different plumage colour traits, often using relatively few museum specimens (Cramp & Simmons, 1980; Priolo, 1984).

It is possible that variability of plumage colours is, at least partially, under environmental nongenetic control, and that phenotypic variation has not been sufficiently sampled by taxonomists. MtDNA data suggest that subspecies *saxatilis* could include the Alpine and eventual north Balkan populations; *graeca* could include central Apennine and central-southern Balkan populations. MtDNA findings do not support the existence of a distinct Apennine subspecies (*A. g. orlandoi*), whereas the distinctiveness of Sicilian *whitakeri* is strongly confirmed.

The mtDNA information obtained in this study should be integrated with data on geographical differentiation of nuclear markers (microsatellites), and rock partridge populations should be managed according to the observed phylogeographical pattern; for example, the presence of three separated units in Italy should be considered: the Alps, Apennines and Sicily. In particular, as partridges are reared in captivity and released for restocking in hunting areas, it is necessary to avoid mixing of stocks of different geographical origins, and, in particular, releasing of continental partridges in Sicily. The red-legged, chukar and rock partridges can easily hybridize in captivity and in nature (Bernard-Laurent & De Franceschi, 1994). It is, therefore, imperative to control the genotypes of released birds and to avoid the illegal use of hybrids.

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