Lack of genetic structure of Cypriot *Alectoris chukar* (Aves, Galliformes) populations as inferred from mtDNA sequencing data

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Abstract

Lack of genetic structure of Cypriot Alectoris chukar *(Aves, Galliformes) populations as inferred from mtDNA sequencing data.*— The Chukar (*Alectoris chukar cypriotes*) is the most common game bird in Cyprus. Since 1990 the Cypriot Government has established a restocking program with captive–reared birds. However, this program has not been guaranteed by checking the genetic nature of wild and farmed samples, either in the areas controlled by the Cypriot Government or in northern Cyprus. The sequencing of both Cytochrome–*b* and Control Region of the mitochondrial DNA was carried out for 61 Cypriot representatives and 14 specimens of the same subspecies from Crete and Israel. Only the *A. chukar* maternal lineage was found. A partitioning of Cypriot specimens among different clades was not reliably supported, whereas robust bootstrap values weighted for an evolutionary divergence between Cypriot and Cretan Chukars. An overall genetic homogeny of the Cypriot populations was disclosed, whatever their status (captive *vs.* wild stocks) and origin (Government controlled *vs.* occupied areas) would be, a higher nucleotide diversity of the wild *vs.* captive representatives notwithstanding.

Key words: Chukar, Control Region, Cytochrome–*b*, Genetic diversity, mtDNA, Partridges.

Resumen

Falta de estructura genética en las poblaciones chipriotas de Alectoris chukar *(Aves, Galliformes), deducida de los datos de secuenciación del ADNmt.*— Una subespecie de la perdiz chucar (*Alectoris chukar cypriotes*) es el ave de caza más común de Chipre. A partir de 1990 el gobierno chipriota estableció un programa de repoblación utilizando aves criadas en cautividad. No obstante, dicho programa no ha sido avalado mediante la comprobación de la naturaleza genética de muestras tanto de ejemplares salvajes como de granja, ni en las zonas controladas por el gobierno chipriota ni en el norte de Chipre. Se ha llevado a cabo la secuenciación del citocromo–*b* y de la región de control del ADN mitocondrial de 61 ejemplares chipriotas y de 14 especimenes de la misma subespecie de Creta y de Israel. Sólo se encontró el linaje materno de *A. chukar*. No se pudo demostrar con fiabilidad el reparto de los especimenes chipriotas en distintos clados, mientras que unos valores bootstrap muy consistentes sustentaban una divergencia evolutiva entre las perdices chucar chipriotas y cretenses. Se reveló la existencia de una homogeneidad genética en las poblaciones chipriotas, cualquiera que fuera su estatus (linajes cautivos frente a salvajes) o su origen (zonas controladas por el gobierno frente a zonas ocupadas), por más que se diera una mayor diversidad de nucleótidos de los ejemplares salvajes frente a los cautivos.

Palabras clave: Perdiz chucar, Región de control, Citocromo–*b*, Diversidad genética, ADNmt, Perdices.

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Introduction

The Chukar partridge (*Alectoris chukar*, Galliformes) is the most common and most popular game bird in Cyprus, the third largest island in the Mediterranean after Sicily and Sardinia. Cyprus also harbours the *A. c. cypriotes* Hartert, a 1,917 subspecies, which is reported to inhabit the Aegean islands (Crete included), southern Turkey and Israel (Madge & McGowan, 2002). Records of the Chukar in Cyprus date back to the Bronze Age (Watson, 1962). It inhabits a wide area, covering rocky habitats with maquis and/or phrygana between the coastal area and the forested peaks of the Troodos Mountains. Cyprus has the largest *A. chukar* population in Europe, with a yearly harvest estimated at 254,000–600,000 hunted birds (1986–2006: P. Panayides, pers. comm.).

Hunting occurs throughout the Chukar's large distribution range that is claimed to extend from the eastern Mediterranean throughout central Asia and up to Manchuria (Madge & McGowan, 2002). Due to its limited range within Europe, *A. chukar* is a Species of European Conservation Concern. The Cypriot population, in particular, is listed as vulnerable (BirdLife International, 2004). The situation was made worse in Cyprus following the severe social–economic changes of 1974, when 37% of the island came under the occupation of Turkey and about 200,000 people were forced to move to the southern region, thus causing considerable stress to wildlife resources in terms of population crowding and land–use changes. Indeed, since that time, Cyprus has experienced a rapid economic growth that has largely contributed to habitat loss because of resort building sprawl, construction of dense road networks, and expansion of agriculture with increased mechanization and pesticide use (World Research Institute, 1992; Panayides, 2005). In addition, there is no doubt that hunting pressure is another important factor accounting for the current status of the Cypriot *A. chukar*, as since the 1970's the number of suitable hunting territories has decreased while the number of hunters has almost doubled (presently close to 50,000: Kassinis, 2001).

In response to these challenges, in 1990 the Cypriot Game Fund Service launched a Chukar release program using local *A. c. cypriotes* birds reared at the Stavrouvoni state farm. A genetic study based on Short Tandem Repeats (STR) markers was recently undertaken to evaluate the situation (Tejedor et al., 2005). Despite its heuristic value, several shortcomings were detected in the above–mentioned study: (i) the number of both the specimens (n = 33) and nuclear loci (n = 4) taken into consideration was a serious constraint, (ii) no accurate geographic records for the studied populations were provided, (iii) no comparison between farmed and wild birds was carried out, and (iv) no specimens from Turkish occupied territory were analysed. The present work employed genetic markers whose effectiveness in comparing genetic relationships among neighbouring and even geographically distant *Alectoris chukar* populations has been consistently proved for both their nature and pair–base lengths: the Cytochrome *b* (Cyt–*b*) and Control Region (CR) markers of the mitochondrial DNA (mtDNA) (Barbanera et al., 2005; Randi et al., 2006; Barbanera et al., in press). Our results support a close genetic relationships between the *A. c. cypriotes* populations and provide insights into the general variation characterizing the species *Alectoris chukar* throughout its range of distribution.

Material and methods

Sampling

Seventy–five *A. chukar cypriotes* specimens were investigated. Samples (liver) were obtained from wild birds (n = 24) hunted in two Government–controlled regions of Cyprus, namely the Alykes coastal wetland area near Larnaca (n = 12) and the mountains of the Paphos Forest (n = 12). Samples of wild birds hunted in the Turkish controlled part of northern Cyprus (Karpas Peninsula, n = 8) were also collected (fig. 1). The above–mentioned populations were originally considered wild, as they have never been restocked with captive specimens (P. Panayides, pers. com.). We also collected samples (feathers) of captive birds (n = 29), obtained from both state (Stavrouvoni, Larnaca District, n = 12) and private game farms (Theodosis: Ayious Trimithias, Nicosia District, n = 6; Sam: Alassa, Limassol District, n = 6; Vorvoromitas, Agrokipia, Nicosia District: n = 5; see fig. 1). Samples obtained from shot birds were carefully collected in order to avoid the analysis of specimens from the same covey. In order to do so, only one sample per hunting trip was kept for genetic analysis. Samples (liver) from *A. chukar* specimens hunted in Crete (Greece, n = 7) and in Israel (n = 7) were also investigated to estimate their possible genetic differentiation with respect to the Cypriot populations. Additional samples from Red–legged Partridge (*Alectoris rufa*, n = 3, liver, Spain), Rock Partridge (*A. graeca*, n = 2, feathers, Italy) and Barbary Partridge (*A. barbara*, n = 1, liver, Italy) were also used as references for the remaining Mediterranean *Alectoris* partridges. The Gene Bank accession codes of the sequences employed in this work are as follows: Cyt–*b*, from AM492908 to AM492953, from AM084553 to AM084575, AJ586141, AJ586147, AJ586150, AJ586157 and AJ586158; CR: from AM084616 to AM084645, from AM492954 to AM492998, AJ586190, AJ586196, AJ586198, AJ586206 and AJ586207.

DNA extraction, PCR amplification and sequencing

The total genomic DNA was extracted from liver fragments using Puregene® Genomic DNA Isolation Kit (Gentra Systems, USA) and from feathers as reported by Barbanera et al. (2005). For each

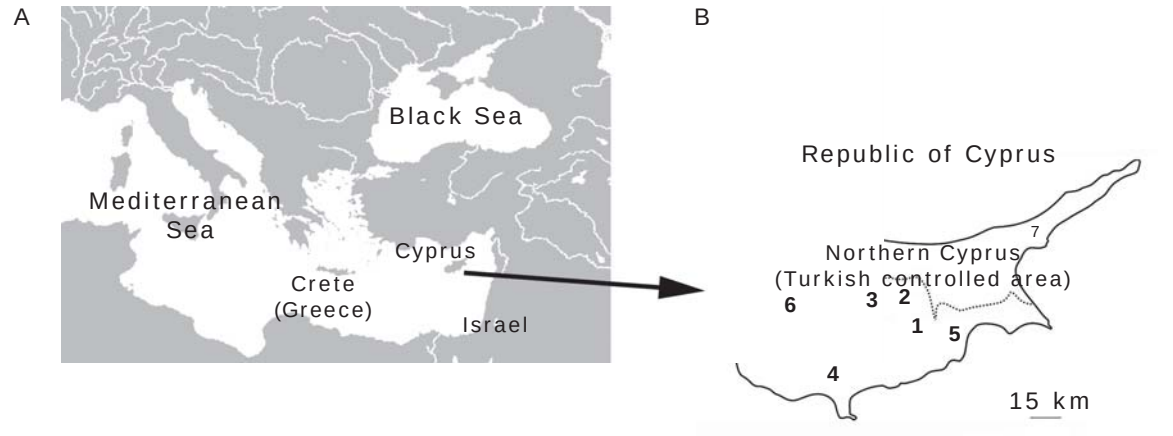

Fig. 1. The studied area in the Mediterranean: A. The relative positions of Crete, Cyprus and Israel are indicated; B. The localities of the sampled Cypriot *A. chukar* populations are indicated (1. Stavrouvoni state farm; 2. Theodosis private farm; 3. Vorvoromitas private farm; 4. Sam private farm; 5. Alykes Larnaca coastal wetland area; 6. Paphos Forest; 7. Karpas Peninsula, in the Turkish occupied territory).

Fig. 1. El área mediterránea estudiada: A. Se indican las situaciones relativas de Creta, Chipre e Israel; B. Se indican las localidades de muestreo de las poblaciones chipriotas de A. chukar*: (1. Granja estatal Stavrouvoni; 2. Granja privada Theodosis; 3. Granja privada Vorvoromitas; 4. Granja privada Sam; 5. Humedal costero Alykes Larnaca; 6. Bosque de Paphos; 7. Península de Karpas, en el territorio ocupado por Turquía.*

specimen almost the entire Cyt–*b* gene (1,092 bp, total length = 1,143 bp) and the whole CR were amplified (Barbanera et al., 2005). PCR products were purified using BioRad Kleen Spin Columns and both DNA strands were directly sequenced on an ABI 310 automated sequencer (Applied Biosystems, USA). In order to assess the authenticity of the mtDNA amplifications, possible nuclear sequences of mitochondrial origin were checked by comparing sequences with those directly obtained from purified mtDNA (Barbanera et al., 2005). Specifically, mtDNA was isolated from the following samples: n. 6, 10, 27 and 42 (*A. chukar*) from Cyprus; n. 4 (*A. chukar*) from Crete; P_{01} (*A. rufa*) from Spain; Uro_{01} (*A. graeca*) and Ier_{03} (*A. barbara*) from Italy.

Phylogenetic data analysis

The alignments of Cyt–*b* and CR sequences from 81 specimens were completed with Clustal (Thompson et al., 1994). The Partition–Homogeneity test as implemented in PAUP* 4.0b10 (Swofford, 2002) was applied to 1,000 replications of an heuristic search with character partitions to determine the statistical validity of combining the two mtDNA genes for the phylogenetic analysis. As the test detected no significant differences among mtDNA gene partitions (P = 0.667), the Cyt–*b* and CR sequences were analysed as combined mtDNA dataset.

The phylogenetic signal was evaluated (i) by means of the index of substitution saturation (Iss, Xia test with 1,000 replicates: Xia et al., 2003) using DAMBE 4.2.13 software (Xia & Xie, 2001), and (ii) by plotting the number of transitions (Ti) and transversions (Tv) against a corrected genetic distance that was obtained by exporting the relative matrix produced by PAUP into Microsoft Excel. Finally, the software MEGA 3 (Kumar et al., 2004) was employed to compute nucleotides composition, genetic distances and nucleotide diversity (π).

Phylogenetic relationships were inferred using both distance and parsimony methods as implemented in PAUP. Concerning the distance method , the model of DNA substitution that best fitted the data was selected using the software FindModel (Los Alamos National Laboratory, New Mexico, USA: http://hcv.lanl.gov/content/hcv–db/findmodel/findmodel.html). FindModel is a weighted neighbour joining–based (NJ) procedure set up by Bruno et al. (2000). It was developed as web implementation of the program Modeltest (Posada & Crandall, 1998). The HKY85 algorithm (Hasegawa et al., 1985) with gamma distribution was then singled out according to the Akaike Information Index criterion (Posada & Buckley, 2004), and the calculation of the α shape parameter was performed as reported by Sullivan et al. (1995). The Maximum parsimony (MP) procedure was set up as follows: unweighted, with TBR swapping algorithm and random addition

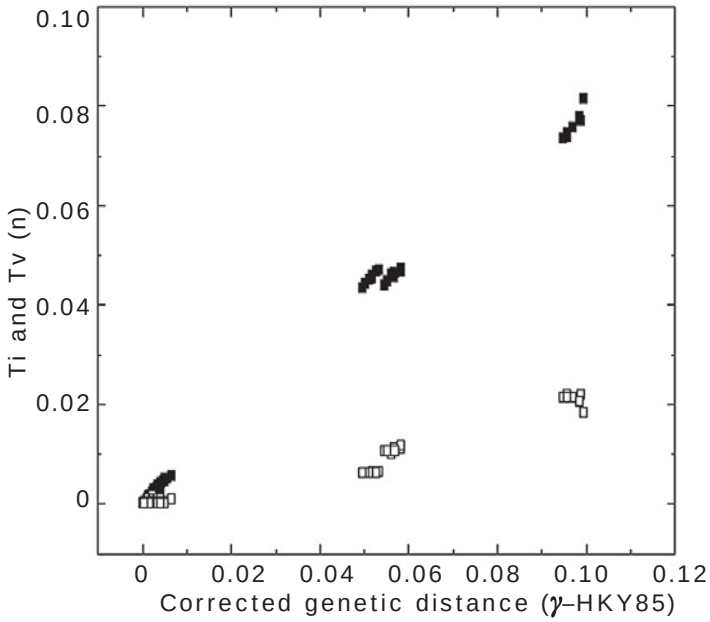

Fig. 2. The number of transitions (ordinates: Ti. Solid square) and transversions (ordinates: Tv. Open square) plotted versus the corrected genetic distance (abscissas: γ–HKY85 evolutionary model).

Fig. 2. Número de transiciones (ordenadas: Ti. Cuadrados sólidos) y transversiones (ordenadas: Tv. Cuadrados huecos) representadas frente a la distancia genética corregida (abscisas: modelo evolutivo γ–HKY85).

sequence limited to 10 rearrangements per bootstrap replicate. Gaps were treated as fifth base. Multiple MP trees were collapsed to obtain a 50% majority–rule consensus tree. For both kinds of reconstruction a Barbary Partridge sequence was employed as outgroup and the statistical support was evaluated by bootstrapping (BP, with 1,000 resampling steps: Felsenstein, 1985).

The partition of the genetic diversity among and within *A. chukar* populations was investigated by means of the analysis of molecular variance (AMOVA, Arlequin 3.01 software package: Excoffier et al., 2005) using pairwise F_{ST} distances of Wright's (1965) *F*–statistics. Distance values were plotted on the first two axes of a Principal Components Analysis (PCA) carried out using the Statistica® ver. 5.0/W Statistica package (Statsoft Inc., USA). The software Arlequin was also used to calculate the haplotype diversity (*h*) and to check for neutral evolution of the investigated mtDNA sequences (Tajima neutrality test).

Results

Phylogenetic data

The alignment of joint Cyt–*b* and CR sequences defined a set of 2,256 characters, indels included (only in the CR). More particularly, the *A. chukar* Cyt–*b* sequences were all 1,092 bp long whereas the CR sequences were all 1,154 bp (cf., Randi & Lucchini, 1998). The real mitochondrial nature of the PCR products obtained from total genomic DNA was assessed by (i) comparison with the sequences from isolated mtDNA, (ii) the detected under–representation of guanine (13.4%), and (iii) the absence of indels and/or internal stop codons (only in the Cyt–*b*).

The analysis of the phylogenetic signal did not disclose any saturation. The average number of Ti was 4.4 higher than Tv, outgroup excluded. In the plot of the number of the Ti and Tv versus divergence measured by the γ–HKY85 evolutionary model, the Ti/Tv ratio was > 1 in the whole distance range (fig. 2). The Iss value (= 0.299) was found to be highly significantly smaller ($P < 10^{-5}$) than that of the critical Iss (Iss.c, the Iss value at which the sequences begin to fail to recover the true tree) assuming either symmetrical or asymmetrical topology for the phylogenetic reconstructions (Iss.c = 0.843 and Iss.c = 0.820, respectively).

Genetic distance analysis with γ–HKY85 algorithm (α = 0.152) produced the NJ tree (92 variable sites) in figure 3. The BP values calculated by MP procedure were added below internodes, as MP produced a 50% majority–rule consensus

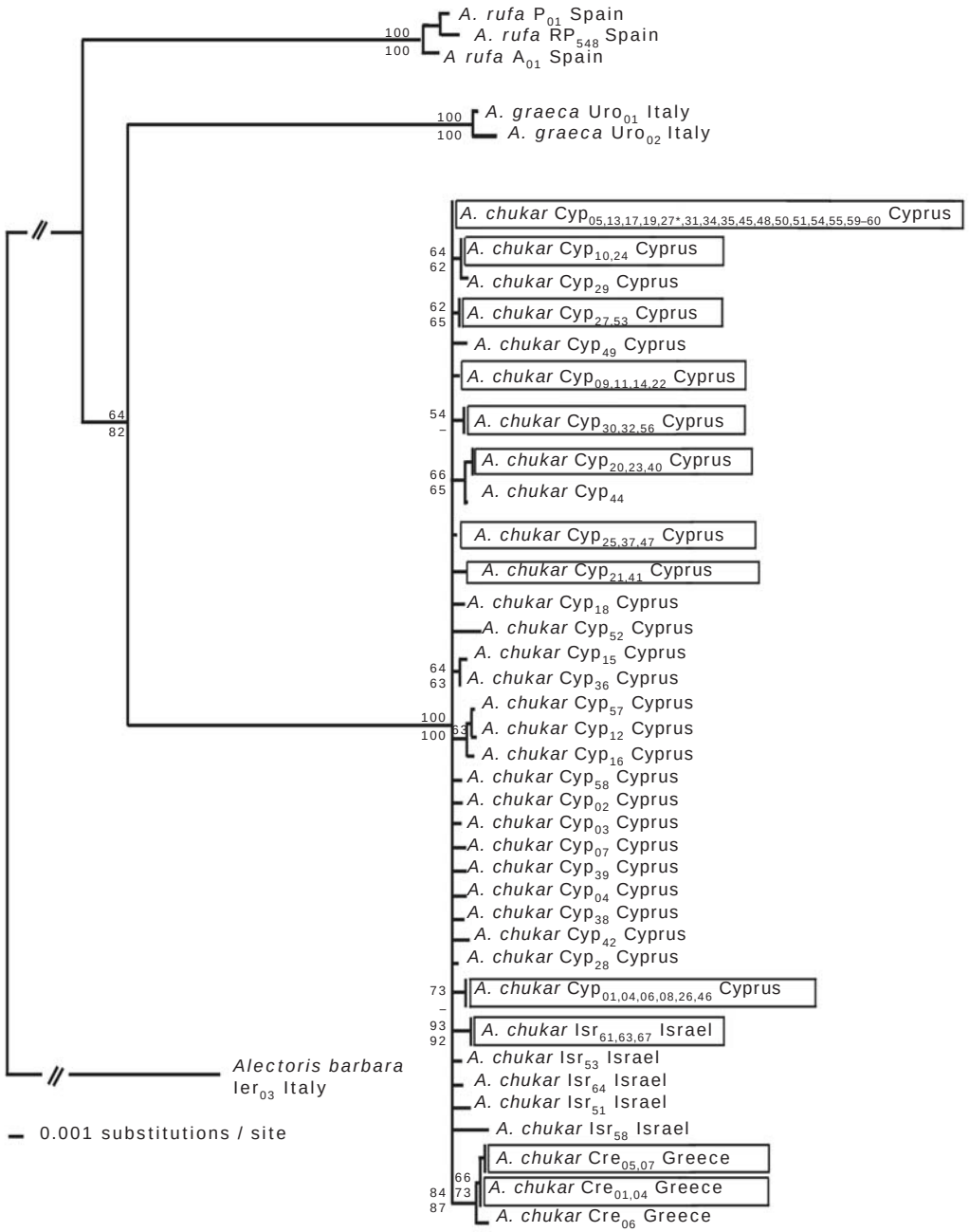

Fig. 3. The neighbour–joining tree computed by PAUP using HKY85 γ–genetic distances (α = 0.152) among the aligned 2,256 characters (indels included) of the joint Cyt–*b* and CR sequences. Numbers at the internodes indicate all the bootstrap percentage values computed in both the NJ (above internodes) and 50% majority–rule consensus MP (below internodes) tree. Rectangular boxes show identical sequences. The phylogenetic trees were rooted using an *A. barbara* sequence. Abbreviations: Cyp. Cyprus; Cre. Crete; Isr. Israel; P_{01}. Mallorca, Spain; A_{01}. Seville, Spain; RP_{548}. Ciudad Real, Spain; $Uro_{01–02}$. Southern Apennines, Italy; Ier_{03}. Sardinia, Italy.

*Fig. 3. Árbol de proximidad calculado mediante PAUP utilizando las distancias genéticas γ–HKY85 (α = 0,152) entre los 2.256 caracteres alineados (incluyendo indels) de las secuencias ensambladas del citocromo–*b *y la región de control. Las cifras de los internodos indican los valores porcentuales bootstrap calculados mediante NJ (por encima de los internodos) y MP de consenso de la regla de la mayoría del 50% (debajo de los internodos). Los rectángulos indican secuencias idénticas. Los árboles filogenéticos se establecieron utilizando una secuencia de* A. barbara*. (Para las abreviaturas ver arriba.)*

tree with the same topology as the NJ tree (152 parsimony informative characters; length, L = 372; consistency index, CI = 0.720; retention index, RI = 0.806). All specimens from Cyprus, Israel and Crete clustered in the same clade (NJ, MP: BP = 100%), showing only the mtDNA lineage corresponding to the Chukar phenotype. Both the *A. rufa* and *A. graeca* clades clearly diverged with respect to the *A. chukar* clade (cf., Randi & Lucchini, 1998). Within the *A. chukar* clade, four clusters supported by BP ranging around 65% and including only Cypriot specimens were found. There was no geographic consistency within these groups, each cluster being formed by specimens from different regions of Cyprus. Most of the *A. chukar* specimens from Israel had a basal position within the clade, with the exception of three specimens clustering apart (*A. chukar* $Isr_{61,63,67}$: NJ, BP = 93%; MP, BP = 92%). All of the specimens from Crete grouped together in the *A. chukar* clade (NJ, BP = 84%; MP, BP = 87%).

Cytochrome–*b* heteroplasmy

A mtDNA heteroplasmic event was disclosed in the Cyt–*b* sequence of the *A. chukar* Cyp_{27} specimen. Amplification was obtained from purified mtDNA and direct sequencing disclosed a double peak on the chromatogram of both DNA strands, revealing a single nucleotide polymorphism (Gene Bank accession codes: AM084572 and AM084573). Indeed, at pos. 504 (cf., Randi, 1996), either guanine or adenine was found (3rd codon pos., no aminoacidic change), the latter being present in all of the Chukars sequenced in this work.

Table 1. Results of the AMOVA analysis for the *A. chukar* populations: Sv. Source of variation (in %, Ap. Among populations, Wp. Within populations); Cy. Cyprus; I. Israel; Cr. Crete.

Tabla 1. Resultados del análisis AMOVA de las poblaciones de A. chukar*: Sv. Origen de la variación (en %, Ap. Entre poblaciones, Wp. Dentro de poblaciones); Cy. Chipre; I. Israel; Cr. Creta.*

	Sv			
Region	Ap	Wp	F_{ST}	*P*
Cy	6.5	93.5	0.065	0.078
Cy + I	22.1	77.9	0.221	$< 10^{-5}$
Cy + Cr	40.5	59.5	0.405	$< 10^{-5}$
Cy + I + Cr	42.3	57.7	0.420	$< 10^{-5}$

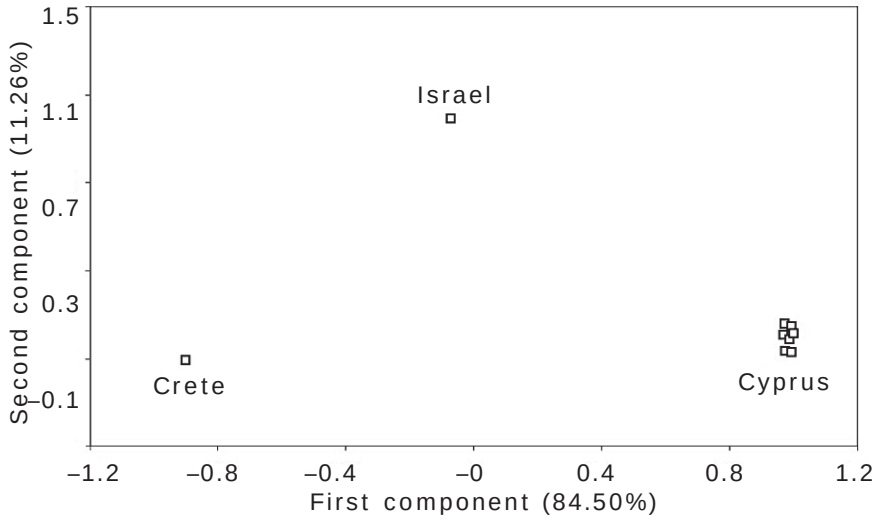

Fig. 4. PCA performed using the pairwise F_{ST} distances calculated for all *A. chukar* ingroups of the phylogenetic reconstructions provided in figure 3. Single specimens were grouped according to each population as reported in table 2.

Fig. 4. PCA llevado a cabo utilizando las distancias F_{ST} en grupos de dos, calculadas para todos los grupos homogéneos de A. chukar *de las reconstrucciones filogenéticos de la figura 3. Los ejemplares aislados se agruparon por poblaciones, tal como se indica en la tabla 2.*

Table 2. Estimates of genetic diversity computed using 2,256 characters of joint Cyt–*b* and CR sequences from nine *A. chukar* populations: PpS. Population sample size; Pl. Polymorphic sites; T. Tajima neutrality test; H. Haplotypes; Uh. Unique haplotypes; Hd. Haplotype diversity; Nd. Nucleotide diversity. (Standard deviation is reported in brackets.)

*Tabla 2. Estimas de la diversidad genética calculadas utilizando 2.256 caracteres de secuencias ensambladas del citocromo–*b *y la región de control de nueve poblaciones de* A. chukar*: PpS. Tamaño de la muestra de la población; Pl. Lugares polimórficos; T. Test de neutralidad de Tajima; H. Haplotipos; Uh. Haplotipos únicos; Hd. Diversidad de haplotipos; Nd. Diversidad de nucleótidos. (La desviación estándar se indica entre paréntesis.)*

	PpS (n)	Pl (n)	T (D; *P*)	H (n)	Uh (n)	Hd (*h*)	Nd (π, %)
Sam farm, Cyprus	6	7	– 1.010; 0.214	4	1	0.800 (0.172)	0.114 (0.044)
Stavrouvoni farm, Cyprus	12	10	– 1.769; 0.013	8	3	0.859 (0.087)	0.081 (0.026)
Theodosis farm, Cyprus	6	5	– 1.337; 0.054	4	1	0.800 (0.172)	0.075 (0.034)
Vorvoromitas farm, Cyprus	5	5	0.273; 0.680	4	2	0.900 (0.161)	0.090 (0.042)
Alykes Larnaca, Cyprus	12	13	– 1.388; 0.082	8	4	0.924 (0.057)	0.130 (0.037)
Paphos Forest, Cyprus	12	8	0.209; 0.620	7	2	0.879 (0.075)	0.125 (0.047)
Karpas Peninsula, Cyprus	8	10	– 0.229; 0.439	7	4	0.964 (0.077)	0.166 (0.051)
Crete, Greece	7	4	– 0.876; 0.267	3	1	0.667 (0.160)	0.060 (0.030)
Israel	7	15	0.225; 0.597	5	4	0.857 (0.137)	0.248 (0.076)

Genetic diversity

As reported in table 1, AMOVA indicated that, when only the Cypriot populations were taken into account, 93.5% of the total genetic variability was distributed within populations, and 6.5% among populations (F_{ST} index = 0.065). When Israeli or Cretan populations were also analysed, the F_{ST} value increased to 0.221 and 0.405, respectively. When all of the *A. chukar* populations were considered, 57.7% of the total genetic variability was distributed within populations, and 42.3% among populations (F_{ST} = 0.420). When the F_{ST} distances calculated for the *A. chukar* sequences were plotted on the first two axes of a PCA (fig. 4), the first two components explained 96% of the total genetic diversity. A significant divergence between the populations from Cyprus, Israel and Crete was disclosed. The genetic distance value calculated between Cypriot and Israeli populations ranged around 0.14%, whereas that between Cypriot and Cretan populations ranged around 0.34%.

The alignment of the *A. chukar* sequences disclosed a total of 35 haplotypes defined by 77 polymorphic sites (table 2). More particularly, 22 haplotypes were unique, most of them (17) being restricted to Cyprus. The haplotype diversity (*h*) was high, its average value ranging around 0.85. The lowest value (*h* = 0.67) showed by the Cretan population was different from those of the other populations (Bonferroni post–hoc multiple comparisons: one way ANOVA, $P < 10^{-5}$ and Bonferroni test, $P < 0.05$). The Israeli population showed the highest value of nucleotide diversity (π = 0.25%: ANOVA, $P < 10^{-5}$; Bonferroni test, $P < 0.05$ versus all populations). Among Cypriot representatives, the nucleotide diversity of the Karpas Peninsula population (π = 0.17%, the highest value) differed significantly from those calculated for the Stavrouvoni, Theodosis and Sam farmed stocks (ANOVA, $P < 10^{-5}$; Bonferroni test, $P < 0.05$). Finally, with the exception of the mtDNA sequences obtained from the Stavrouvoni population, all the others evolved neutrally (Tajima's D: table 2).

Discussion

A great number of travellers' notes reported that Chukar was abundant in the eastern Mediterranean until the 19th century. Nowadays, Chukar populations have disappeared from several Greek islands. There are a few exceptions, but surviving populations are small and suffering decline due to abandonment of traditional wheat crops, illegal hunting and habitat disappearance or deterioration (Papavangelou et al., 2001). Furthermore, genetic studies dealing with Mediterranean *A. chukar* populations are scarce (Tejedor et al., 2005; Barbanera et al., in press) when compared to those referring to *A. rufa* and *A. graeca*, the only exception being the *A. chukar* populations colonizing Israel (see Randi et al.,

2006 for a review). Disappointingly, such a poor genetic knowledge is potentially harmful for the conservation of the species. As discussed by Frankham (2005), it can lead to misguided management plans because of the risk of introduction of non–endemic, invasive genotypes or subspecies through restocking with captive stocks.

Since 1990 the Cypriot *A. c. cypriotes* populations have been subjected to restocking by the Game Fund (Ministry of Interior, Cyprus). Such a release program originally involved captive stock from Stavrouvoni state farm that originated from eggs collected in the wild. The program was later extended to a restricted number of private farms, which started to rear Chukars under the supervision of the Game Fund. A 2,256 character dataset provided by sequencing of Cyt–*b* and CR mtDNA markers from 75 partridges that showed the outwardly identifying features of the *A. chukar* species, was analysed to gain insight into the genetic kinship of Cypriot, Cretan and Israeli populations. According to the "total evidence" approach stating that the phylogenetic analysis should be performed on a combined dataset using all of the possible evidence (Kluge & Wolf, 1993), the tree reconstruction was accomplished using previously jointed Cyt–*b* and CR sequences. The results showed a flawless correlation between the mtDNA lineage and the outward Chukar appearance of the analyzed representatives (fig. 3). Taking into account that anthropogenic hybridization between *A. chukar* and *A. rufa* is a widespread phenomenon even in small islands, whatever the parental origin of the introgressive swarms could be (Barbanera et al., 2005; Barbanera et al., in press), the absence of allochthonous mtDNA lineages is a promising result for the conservation of the native *A. chukar* genome in the eastern Mediterranean. Further, no robust genetic differentiation between farmed and wild Cypriot populations was found (BP < 70%, fig. 3; cf., Felsestein, 2004), also when specimens from north Cyprus were considered. Within the *A. chukar* clade, only a few Israeli representatives clearly clustered apart from the remaining ingroups (BP > 90%, fig. 3). This might be related to the known genetic differentiation due to geographical isolation of Israeli Chukar populations occurring across the north Negev ecotone (Randi et al., 2006).

The AMOVA analysis of the Cypriot *A. chukar* showed that the very large majority of the genetic variability was distributed among individuals, the populations thus representing a largely homogeneous group. Only when Cretan and Israeli Chukars were also taken into account in the analysis, was an overall, significant divergence found (table 1). On one hand, the differentiation that was disclosed between Cypriot and Cretan populations (figs. 3, 4: average genetic distance = 0.34%) was in agreement with the morphological evidence reporting that Cretan *A. c. cypriotes* specimens are smaller and darker than Cypriot specimens (Cramp & Simmons, 1980). Such a slightly variant phenotype has accounted for the past assignation of the Cretan population to the *A. c. scotti* subspecies, which is no longer considered valid taxon (Madge & McGowan, 2002). On the other hand, the differentiation between Israeli and Cypriot populations (table 1; average genetic distance = 0.14%) was likely due to the robust divergence showed by a few Israeli representatives coupled to the limited size of the relative sampling (figs. 3, 4). Hence, a deeper investigation is needed to definitively clarify the genetic relationships between Cypriot and Israeli *A. chukar.*

The genetic information was gained from the total number of haplotypes (n = 35, unique haplotypes = 22; table 2), which was comparable to that found in a similar work where several Israeli *A. chukar* populations were analyzed (Randi et al., 2006). However, this result was obtained with a lower number of samples and a longer segment of sequenced mtDNA (Randi et al., 2006: 216 samples from five populations, 444 characters, only CR; this study: 75 samples from nine populations, 2,256 characters, Cyt–*b* and CR). Moreover, in the present work, a single nucleotide polymorphism for the Cyt–*b* sequence of the *A. chukar* Cyp_{27} specimen was found. To our knowledge, this represents the first case of mtDNA heteroplasmy for a coding gene in birds, the other event recorded in the literature dealing with the CR sequence of a razorbill (*Alca torda*: Moum & Bakke, 2001). To sum up, the Cypriot populations showed a large haplotype diversity that, however, did not differ significantly from the Israeli representatives. Although the nucleotide diversity of the wild Cypriot populations was significantly higher than that disclosed in the captive birds, the whole range of detected variation (between 0.07%, Theodosis farm population, and 0.17%, Karpas Peninsula population) dealt with very low percentages (table 2). Hence, the high rates of the haplotype diversity coupled to the low values of the nucleotide diversity confirmed once more the genetic homogeny of the Cypriot populations.

The data provided in this work suggest that Cypriot *A. chukar* should be considered as a single unit for conservation purposes; whether their representatives (i) are captive or wild, (ii) inhabit southern or northern part of the island, or (iii) belong or not to restocked populations does not appear to be of relevance. Although the genetic markers used in the present work follow a maternal inheritance, our results support a high rate of genetic flux among the studied Chukar populations throughout the entire island of Cyprus (cf., Tejedor et al., 2005). This, in turn, might be related to the actions undertaken by the Cypriot Government to preserve the local *A. chukar* breeding stock, such as, for instance, the creation of well–distributed game reserves working as breeding banks. These areas, where hunting is strictly forbidden, occupy 30% of Government–controlled territory. Nevertheless, the value of the nucleotide diversity index estimated for the wild populations was found to be higher than that calculated for the captive populations. Conventional wis-

dom suggests that a regular renewal of the captive breeding stocks by means of selected drawing from wild populations would not be just an academic exercise. Finally, the occurrence of genetic differentiation particularly between Cypriot and Cretan *A. chukar* populations suggests that translocation of birds throughout the eastern Mediterranean should not be recommended, although Cyprus and southern Aegean islands officially harbour the same subspecies (Madge & McGowan, 2002). In conclusion, a large data set will hopefully be available following the STR markers–based study that the University of Pisa is developing in collaboration with the Cypriot Ministry of the Interior, the Greek Hunting Federation of Macedonia–Thrace and the Bahauddin Zakariya University (Multan, Pakistan). The goal of this study is the identification of both Mediterranean and Asian *A. chukar* populations in order to bring the exchange of commercial stocks into line with knowledge of the genetic kinship, thus conferring to their management the status of a scientific process.

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References

Barbanera, F., Negro, J. J., Di Giuseppe, G., Bertoncini, F., Cappelli, F. & Dini, F., 2005. Analysis of the genetic structure of red–legged partridge (*Alectoris rufa*, Galliformes) populations by means of mitochondrial DNA and RAPD markers: a study from central Italy. *Biological Conservation*, 122: 275–287.

Barbanera, F., Guerrini, M., Hadjigerou, P., Panayides, P., Sokos, P., Wilkinson, P., Khan, A. A., Khan, B. Y., Cappelli, F. & Dini, F. (in press). Genetic insight into Mediterranean chukar (*Alectoris chukar*, Galliformes) populations inferred from mitochondrial DNA and RAPD markers. *Genetica*.

Birdlife International, 2004. *Birds in Europe: population estimates, trends and conservation status*. BirdLife Conservation Series, vol. 12. BirdLife International, Wageningen, The Netherlands.

Bruno, W. J., Socci, N. D. & Halpern, A. L., 2000. Weighted Neighbor Joining: A Likelihood–Based Approach to Distance–Based Phylogeny Reconstruction. *Molecular Biology and Evolution*, 17: 189–197.

Cramp, S. & Simmons, K. E. L., 1980. *Handbook of the Birds of Europe, the Middle East and North Africa, vol. 2, The Birds of the Western Paleartic*. Oxford Univ. Press, Oxford.

Excoffier, L., Laval, G. & Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1: 47–50.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783–791.

– 2004. *Inferring Phylogenies*. Sinauer Associates, Inc. Publishers Sunderland, Massachusetts.

Frankham, R., 2005. Genetics and extinction. *Biological Conservation,* 126: 131–140.

Hasegawa, M., Kishino, H., Yano, T.–A., 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. J*ournal of Molecular Evolution*, 22: 160–174.

Kassinis, N., 2001. Chukar partridge status and conservation in Cyprus. In: *Proceedings of the Symposium on the status, management and conservation of the species Alectoris, Black Francolin, Thrush, Quail and Turtle Dove in the Mediterranean Region:* 84–89 (N. Kassinis & P. Panayides, Eds). The Cypriot Game Fund Service, Ministry of Interior, Nicosia, Cyprus.

Kluge, A. G. & Wolf, A. J., 1993. Cladistics: What's in a word ? *Cladistics*, 9: 183–199.

Kumar, S., Tamura, K. & Nei, M., 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Briefings in Bioinformatics*, 5: 150–163.

Madge, S. & McGowan, P., 2002. *Pheasants, partridges and grouse*. A and C Black Ltd., London.

Moum, T. & Bakke, I., 2001. Mitochondrial control region structure and single site heteroplasmy in the razorbill (*Alca torda*; Aves). *Current Genetics*, 39: 198–203.

Panayides, P., 2005. Six Aspects of Land Use and Development Activity that Result in Adverse Effects to Cypriot Wildlife Resources. In: *Proceedings of the XXVth International Congress of the International Union of Game Biologists and the IXth Symposium Perdix:* 76–90 (E. Hadjisterkotis, Ed.). Government Printing Office, Ministry of Interior, Cyprus.

Papaevangelou, E., Thomaides, C., Handrinos, G. & Haralambides, A., 2001. Status of partridges (*Alectoris* and *Perdix*) species in Greece. *Game and Wildlife Science*, 18**:** 253–260.

Posada, D. & Crandall, K. A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14: 817–818.

Posada, D. & Buckley, T. R., 2004. Model selection and model averaging in phylogenetics: advan-

tages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, 53: 793–808.

Randi, E., 1996. A Mitochondrial Cytochrome *B* phylogeny of the *Alectoris* Partridges. *Molecular Phylogenetics and Evolution*, 2: 214–227.

Randi, E. & Lucchini, V., 1998. Organization and evolution of the Mitochondrial DNA Control Region in the Avian Genus *Alectoris*. *Journal of Molecular Evolution*, 47: 449–462.

Randi, E., Tabarroni, C. & Kark, S., 2006. The role of history vs. demography in shaping genetic population structure across an ecotone: chukar partridges (*Alectoris chukar*) as a case study. *Diversity and Distributions*, 12: 714–724.

Sullivan, J., Holsinger, K. E. & Simon, C., 1995. Among–Site Rate Variation and Phylogenetic Analysis of 12S rRNA in Sigmodontine Rodents. *Molecular Biology and Evolution*, 12: 988–1001.

Swofford, D. L., 2002. *PAUP*: Phylogenetic Analysis Using Parsimony*. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tejedor, M. T., Monteagudo, L. V., Hadjisterkotis, E. & Arruga, M. V., 2005. Genetic variability and population structure in Cypriot chukar partridges (*Alectoris chukar cypriotes*) as determined by microsatellite analysis. *European Journal of Wildlife Research*, 51: 232–236.

Thompson, J. D., Higgins, D. G. & Gibson, T. J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions–specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4673–4680.

Watson, G. E., 1962. Three sibling species of *Alectoris* partridge. *Ibis*, 104: 353–367.

World Resources Institute, 1992. *Environmental almanac*. Houghton Mifflin Company. Boston, Massachusetts.

Wright, S., 1965. The interpretation of population structure by *F*–statistics with special regards to systems of mating. *Evolution*, 19: 395–420.

Xia, X. & Xie, Z., 2001. DAMBE: Data analysis in molecular biology and evolution. *Journal of Heredity*, 92: 371–373.

Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y., 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution*, 26: 1–7.