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ORIGINAL PAPER

Genetic structure of Mediterranean chukar (*Alectoris chukar*, Galliformes) populations: conservation and management implications

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Abstract The chukar (*Alectoris chukar*, Galliformes) is a species hunted throughout its native range from the East Mediterranean to Manchuria and in the USA, which hosts the world's largest introduced population. This study aims to investigate the genetic structure of Mediterranean chukar populations to aid management decisions. We genotyped 143 specimens at two regions of the mitochondrial DNA (mtDNA: cytochrome *b*, control region) and eight loci of the microsatellite DNA. Samples were collected in northern (Limnos, Lesvos, Chios) and southern (Crete) Aegean islands (Greece) and Cyprus. We also carried out mtDNA-based comparison with chukars (n=124) from Asia (16 countries) and the USA (five states). We propose six management units for Mediterranean populations. Given their genetic integrity, Limnos and Cyprus, which host different subspecies, proved to be of primary conservation interest. We found exotic *A. chukar* mtDNA lineages in Lesvos, Chios and Crete and produced definitive genetic evidence for the Asian origin of the US chukars.

Keywords Captive-reared birds · Evolutionarily significant unit · Management unit · Microsatellite DNA · Mitochondrial DNA · Partridges

The knowledge of the genetic structure of wild populations is essential for their management and for the selection of those worthy of protection, as limited funds may preclude active conservation for all units (Allendorf and Luikart 2007). This can be a challenging and worthwhile task as the protection of distinct populations helps to maintain high level of biodiversity thus minimising extinction risks (Waples 1991).

Ryder (1986) introduced the concept of evolutionarily significant unit (ESU) for prioritising units below recognised taxonomical levels, given that existing classification may not reflect underlying genetic diversity. Moritz (1994) stressed reciprocal monophyly and divergence of allele frequency at mitochondrial and nuclear DNA loci, respectively, as the distinctive ESU's attribute. Despite the subsequent ESU definitions (Crandall et al. 2000; Kizirian and Donnelly 2004; Degner et al. 2007), genetic data always play a critical role for identifying evolutionary units for conservation purposes. Establishing management units (MUs or demographically independent populations) represent another important conservationist tool to preserve local diversity (Palsbøll et al. 2007). However, allele frequency differentiation on its own is not evidence of demographic independence, which can be assessed by estimating the

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dispersal rate among populations. Hastings (1993) indicated that the dispersal rate among independent populations should be lower than 10% of their effective size (N_e).

In the order Galliformes, socio-economic reasons associated with hunting require a number of birds for shooting that can be met only by artificial rearing. Huge releases of farmed birds are raising fears that local adaptations of wild populations might be disrupted, resulting in population decline and loss of biodiversity (Randi 2008). The practise of releasing captive-reared birds just before ("put and take": Byers and Burger 1979) or after a hunting season is spreading throughout Europe and Asia, whereas it has decreased in the USA, being proven ineffective to sustain wild populations (cf., Sokos et al. 2008).

The chukar (*Alectoris chukar*, Galliformes) is a species hunted throughout its native range, which extends from the Balkans across central Asia up to Manchuria, as well as in the USA, which hosts the world's largest introduced population (Madge and McGowan 2002). This and other factors warranted inclusion of the chukar in the directory of Species of European Conservation Concern (BirdLife International 2004), even though it is listed as Least Concern by the International Union for Conservation of Nature. Different to other *Alectoris* species such as the red-legged partridge (*Alectoris rufa*), hybridisation is not a major threat to the conservation of chukar. In the wild, chukar hybridises only in narrow areas: with *Alectoris graeca* (rock partridge) at the border between Bulgaria and Greece (Dragoev 1974) and with *Alectoris magna* (rusty-necklaced partridge) in the Liu-pan mountains (China: Liu et al. 2006). In captivity, chukar is the most prolific *Alectoris* breeder and crossing with any congeneric species to restock with the former would decrease its fitness (Barbanera et al. 2007).

We investigated the genetic structure of five Mediterranean chukar populations to aid management decisions. As artificially reared chukars are globally traded and released in the wild, genetic monitoring of both wild and captive resources is necessary to plan conservation actions. We used either mitochondrial (mtDNA) or microsatellite (short tandem repeats, STR) DNA markers due to their complementary nature essential to properly address our goal (Ballard and Whitlock 2004; Godinho et al. 2008).

Material and methods

Study area

Two chukar subspecies inhabit the Mediterranean: *A. chukar kleini* Hartert, 1925, occurs in South Bulgaria, North East Greece, North Turkey and North Aegean islands, whereas *A. chukar cypriotes* Hartert, 1917, is found on South Aegean islands, South Turkey and Cyprus (Madge and McGowan 2002). We studied chukars from the Greek islands of Limnos, Lesvos and Chios in the North Aegean, Crete in the South Aegean and Cyprus in the East Mediterranean (Electronic Supplementary Material S1).

Chukars were imported by man to Mediterranean islands for dietary needs not later than 4,000 years ago (Masseti 1997). Very little is known about these populations. Releases of chukar from the Greek mainland have occurred in the past on Lesvos, Chios and Crete. Since the late 1990s, the Greek Forest Service Game Farm at Rethymno (Crete) started to work with local birds. On Limnos, there are no game farms and the import of chukars has never occurred (C. Sokos personal communication, 2008). Cyprus hosts the largest chukar population in Europe, with a yearly harvest of 250,000–500,000 hunted birds. In 1990, the Game Fund Service launched a release programme using chukars from the government farm of Stavrouvoni. Little is known about the management in the Turkish-occupied part of Cyprus (Panayides 2005).

Sampling

Crete and Cyprus required a wider sampling because of their larger size. We collected 143 chukar samples between 2004 and 2007 (S2). Liver fragments came from hunted birds and feather samples from specimens kept in the Stavrouvoni (Cyprus) and Rethymno (Crete) farms. Thirteen samples (liver) of Cretan chukars collected in 2006 were provided by The Natural History Museum of Crete. These 143 samples were investigated by means of both mtDNA and STR markers. Then, we compared (only mtDNA) Mediterranean chukars with 124 allopatric conspecifics from Asia (16 countries, n=106) and USA (five states, n=18). Finally, an Italian rock partridge was used as out-group in the phylogenetic reconstructions (total mtDNA sample size $143 + 124 + 1 = 268$: see S2 for details).

DNA extraction

DNA was extracted from liver and blood using the Puregene Core Kit-a (Qiagen, Germany) following the manufacturer's instructions and from feathers as in Barbanera et al. (2005).

mtDNA

We amplified the partial cytochrome *b* (Cyt-*b*, 1,092 bp; total length, 1,143 bp) and the entire control region (CR, approximately 1,155 bp) of the mtDNA as in Barbanera et al. (2005). We purified and sequenced polymerase chain reaction (PCR) products as in Barbanera et al. (2009a). Hence, we aligned 268 combined sequences (Cyt-*b* + CR:

partition-homogeneity test, P=0.68, PAUP* 4.0b10: Swofford 2002) using CLUSTALW 1.81 (Thompson et al. 1994). We used PAUP* to infer phylogenetic relationships with both neighbour joining (NJ: Saitou and Nei 1987) and maximum parsimony (MP: Swofford et al. 1996) methods. We selected the transitional evolutionary model (TIM + I + G, with: A=0.27; C=0.31; G=0.13; T=0.29; I=0.83; α=0.831) using MODELTEST 3.6 (Posada and Crandall 1998) and the Akaike Information Criterion (Posada and Buckley 2004). We set up the MP procedure following Barbanera et al. (2007) and performed a maximum likelihood (ML) analysis under the general time reversible (GTR) model (with the rate matrix produced by MODELTEST: a=1.0; b=78.4; c=3.9; d=3.9; e=39.8; f=1.0) using a quartet puzzling procedure (10,000 steps) with TREE-PUZZLE 5.2 (Strimmer and von Haeseler 1996). The statistical support was evaluated by bootstrapping (BP, 1,000 replicates: Felsenstein 1985). The haplotype (H) sequences were deposited at the Gene Bank (FM203125–FM203227).

We investigated the partition of the mtDNA diversity among and within all chukar populations by analysis of molecular variance (AMOVA) with ARLEQUIN 3.01 (Excoffier et al. 2005) using φ_{ST} analogous of Wright's (1965) F statistics (1,000 permutations). The φ_{ST} distance values were plotted on the first two axes of a principal component analysis (PCA) using STATISTICA 5.0/W (Statsoft Inc., USA). ARLEQUIN was used to calculate the number of polymorphic sites, the nucleotide diversity (π), the number of pairwise differences (k) and the haplotype diversity (h). Allochthonous haplotypes were not included in the analyses in order to estimate only the native genetic diversity. We estimated gene flow among Cypriot populations with DNASP 4.10 (Rozas et al. 2003) by calculating the effective number of migrants per generation (N_em) using the N_{ST} estimator of Lynch and Crease (1990).

STR

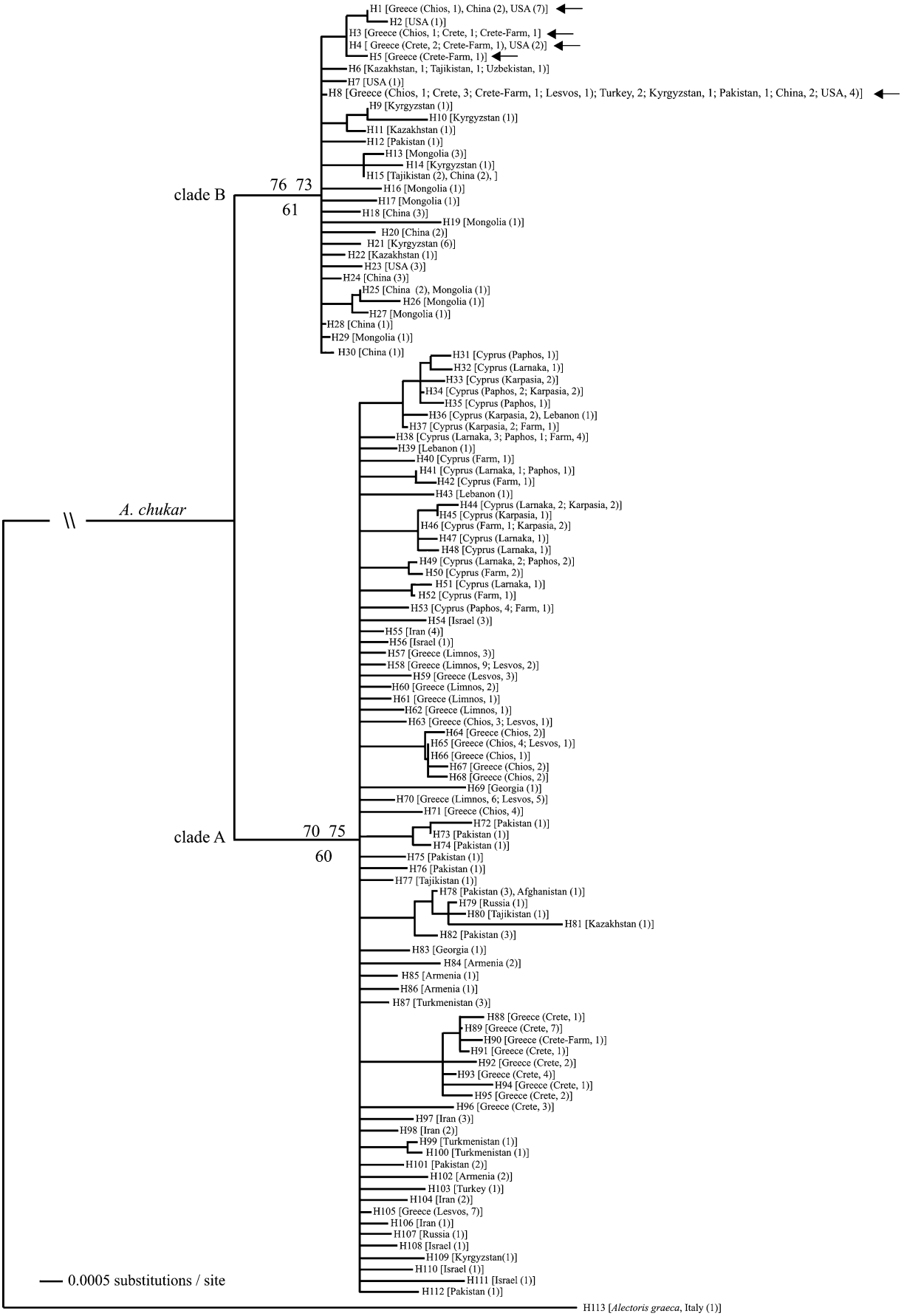
We genotyped chukars (n=143) at eight STR loci using primers (S3) isolated from chicken (*Gallus gallus*) and red-legged partridge (*A. rufa*) genome (Gonzalez et al. 2005; Barilani et al. 2007). PCR reactions (12.5 µl) contained 10 ng of DNA, 2 mM $MgCl_2$, 2 mM dNTP, 0.6 µM of each primer, 1× PCR Gold buffer and 0.3 U/µl Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, USA). Thermal profile was 94°C for 10 min, then five cycles of 45 s at 94°C, 45 s at the first annealing temperature, and 1 min at 72°C, 25 cycles of 45 s at 94°C, 45 s at the second annealing temperature, and 1 min at 72°C, with a final extension at 72°C for 10 min (S3). We used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to assess the quality of the STR scoring. We computed the number of alleles per locus, the number of unique alleles, the allelic richness and the Nei's index (I_N) with FSTAT 2.9.3 (Goudet 2001). We used GENEPOP 3.4 (Raymond and Rousset 1995) to infer deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LE). The significance level of HWE and LE test was estimated using the Bonferroni correction (Hochberg 1988).

We investigated the partition of the STR diversity among and within all chukar populations by AMOVA with ARLEQUIN using pairwise F_{ST} distances. The values were plotted on the axes of a factorial correspondence analysis (FCA) using GENETIX 4.02 (Belkhir et al. 2001). We evaluated population structure using Bayesian cluster analysis with STRUCTURE 2.1 (Pritchard et al. 2000). We carried out Markov chain Monte Carlo simulations without either prior information on the origin of samples or admixture model. We assumed that the maximum number of populations (K) varied between one and nine. For each value of K, we performed ten replicates and set the number of steps for burn in and simulations to 20,000 and 100,000, respectively. We chose the correct K value using the maximum of the function $\Delta K = m(|L(K+1) - 2L(K) + L(K-1)|)/s[L(K)]$, where $L(K)$ stands for "log estimated likelihood" calculated for each K value, m for "mean" and s for "standard deviation" (Evanno et al. 2005). We carried out another Bayesian analysis employing either the individuals not assigned or those that did not match their own sampling population (cluster identification threshold, q_i=0.90: Vaha and Primmer 2006). We tested not-assigned specimens against the putative populations of origin using the PopFlag option (admixture model, 20,000/100,000 steps). This analysis was more powerful in clarifying assignment as the allele frequency for each population was calculated from indubitably assigned samples instead of being estimated as in the first Bayesian analysis. The gene flow among Cypriot populations was estimated via the private allele method (Slatkin 1985) using GENEPOP.

Results

mtDNA

The alignment (out-group included) defined a set of 2,248 characters, indels included; 113 haplotypes were found (S2). The NJ, MP (length, 363; consistency index, 0.478; retention index, 0.703) and ML trees concurrently clustered the haplotypes into two groups (Fig. 1). Henceforth, we reported the BP values in the following order: NJ, MP and ML. The clade A (BP 70/75/60) included all specimens from Cyprus, the large majority of the Greek islands and from the Mediterranean to central Asia. The clade B (BP 76/73/61) comprised all remaining individuals including a


H1 [Greece (Chios, 1), China (2), USA (7)]
H2 [USA (1)]
H3 [Greece (Chios, 1; Crete, 1; Crete-Farm, 1]
H4 [Greece (Crete, 2; Crete-Farm, 1), USA (2)]
H5 [Greece (Crete-Farm, 1)]
H6 [Kazakhstan, 1; Tajikistan, 1; Uzbekistan, 1)]
H7 [USA (1)]
H8 [Greece (Chios, 1; Crete, 3; Crete-Farm, 1; Lesvos, 1); Turkey, 2; Kyrgyzstan, 1; Pakistan, 1; China, 2; USA, 4)]
H9 [Kyrgyzstan (1)]
H10 [Kyrgyzstan (1)]
H11 [Kazakhstan (1)]
H12 [Pakistan (1)]
H13 [Mongolia (3)]
H14 [Kyrgyzstan (1)]
H15 [Tajikistan (2), China (2),]
clade B
76 73
61
H16 [Mongolia (1)]
H17 [Mongolia (1)]
H18 [China (3)]
H19 [Mongolia (1)]
H20 [China (2)]
H21 [Kyrgyzstan (6)]
H22 [Kazakhstan (1)]
H23 [USA (3)]
H24 [China (3)]
H25 [China (2), Mongolia (1)]
H26 [Mongolia (1)]
H27 [Mongolia (1)]
H28 [China (1)]
H29 [Mongolia (1)]
H30 [China (1)]
H31 [Cyprus (Paphos, 1)]
H32 [Cyprus (Larnaka, 1)]
H33 [Cyprus (Karpasia, 2)]
H34 [Cyprus (Paphos, 2; Karpasia, 2)]
H35 [Cyprus (Paphos, 1)]
H36 [Cyprus (Karpasia, 2), Lebanon (1)]
H37 [Cyprus (Karpasia, 2; Farm, 1)]
H38 [Cyprus (Larnaka, 3; Paphos, 1; Farm, 4)]
H39 [Lebanon (1)]
H40 [Cyprus (Farm, 1)]
H41 [Cyprus (Larnaka, 1; Paphos, 1)]
H42 [Cyprus (Farm, 1)]
H43 [Lebanon (1)]
A. chukar
H44 [Cyprus (Larnaka, 2; Karpasia, 2)]
H45 [Cyprus (Karpasia, 1)]
H46 [Cyprus (Farm, 1; Karpasia, 2)]
H47 [Cyprus (Larnaka, 1)]
H48 [Cyprus (Larnaka, 1)]
H49 [Cyprus (Larnaka, 2; Paphos, 2)]
H50 [Cyprus (Farm, 2)]
H51 [Cyprus (Larnaka, 1)]
H52 [Cyprus (Farm, 1)]
H53 [Cyprus (Paphos, 4; Farm, 1)]
H54 [Israel (3)]
H55 [Iran (4)]
H56 [Israel (1)]
H57 [Greece (Limnos, 3)]
H58 [Greece (Limnos, 9; Lesvos, 2)]
H59 [Greece (Lesvos, 3)]
H60 [Greece (Limnos, 2)]
H61 [Greece (Limnos, 1)]
H62 [Greece (Limnos, 1)]
H63 [Greece (Chios, 3; Lesvos, 1)]
H64 [Greece (Chios, 2)]
H65 [Greece (Chios, 4; Lesvos, 1)]
H66 [Greece (Chios, 1)]
H67 [Greece (Chios, 2)]
H68 [Greece (Chios, 2)]
H69 [Georgia (1)]
H70 [Greece (Limnos, 6; Lesvos, 5)]
H71 [Greece (Chios, 4)]
clade A
70 75
60
H72 [Pakistan (1)]
H73 [Pakistan (1)]
H74 [Pakistan (1)]
H75 [Pakistan (1)]
H76 [Pakistan (1)]
H77 [Tajikistan (1)]
H78 [Pakistan (3), Afghanistan (1)]
H79 [Russia (1)]
H80 [Tajikistan (1)]
H81 [Kazakhstan (1)]
H82 [Pakistan (3)]
H83 [Georgia (1)]
H84 [Armenia (2)]
H85 [Armenia (1)]
H86 [Armenia (1)]
H87 [Turkmenistan (3)]
H88 [Greece (Crete, 1)]
H89 [Greece (Crete, 7)]
H90 [Greece (Crete-Farm, 1)]
H91 [Greece (Crete, 1)]
H92 [Greece (Crete, 2)]
H93 [Greece (Crete, 4)]
H94 [Greece (Crete, 1)]
H95 [Greece (Crete, 2)]
H96 [Greece (Crete, 3)]
H97 [Iran (3)]
H98 [Iran (2)]
H99 [Turkmenistan (1)]
H100 [Turkmenistan (1)]
H101 [Pakistan (2)]
H102 [Armenia (2)]
H103 [Turkey (1)]
H104 [Iran (2)]
H105 [Greece (Lesvos, 7)]
H106 [Iran (1)]
H107 [Russia (1)]
H108 [Israel (1)]
H109 [Kyrgyzstan(1)]
H110 [Israel (1)]
H111 [Israel (1)]
H112 [Pakistan (1)]
0.0005 substitutions / site
H113 [Alectoris graeca, Italy (1)]

◀ **Fig. 1** The NJ tree computed by PAUP* for the aligned 113 haplotypes using the TIM + I + G algorithm (I=0.83; α=0.831). *Numbers* at the *internodes* indicate BP values computed in the NJ (*above internodes*), MP (*below internodes*, to the *left*) and ML (*below internodes*, to the *right*) trees. An *A. graeca* specimen was used as out-group (H113). Chukar haplotype (H1–H112: see S2 for details) total occurrence and sampling area are reported. *Black arrows* indicate haplotypes of East Asia origin found in Mediterranean chukars (Greece, Turkey). Abbreviations: *Cyprus-Paphos*, Paphos forest; *Cyprus-Larnaka*, Larnaka coastal area; *Cyprus-Karpasia*, Karpasia; *Cyprus-Farm*, Stavrouvoni farm; *Crete-Farm*, Rethymno farm

few from Lesvos (n=1), Chios (n=3), Crete (wild: n=6; captive, n=4) and Turkey (n=2). All chukars from East Asia and USA hold the clade B (eastern) haplotypes, the clade A (western) ones being distributed from the Mediterranean to central Asia. Chios, Lesvos, Crete and Turkey showed haplotypes of either A or B type (see S4). We did not find any chukars with mtDNA not corresponding to their phenotype.

The AMOVA results were reported in Table 1. The φ_{ST} values showed that most of the Greek and Cypriot pairs were significantly differentiated among themselves and with respect to most of the other populations (Table 2). When the φ_{ST} values were plotted on a PCA, the first two axes explained 81.4% of the total diversity. The populations ranging from the Mediterranean to central Asia diverged with respect to those from East Asia and USA (S5).

Haplotype diversity, nucleotide diversity and mean number of pairwise differences of the Greek and Cypriot populations did not significantly differ from each other, yet these latter showed the highest values (Table 3, S6). Gene flow (N_em) was: Paphos-Larnaka=19.7; Paphos-Farm=10.3, Paphos-Karpasia=12.2; Farm-Karpasia=5.5; others were null.

STR data

The scoring quality of the STR loci was satisfactory because no allelic dropout was present and only 3.5% of the microsatellite locus–population combinations (40 out of 143×8=1,144) gave evidence of null alleles. The average values of H_O were smaller than H_E for each population except Crete-Farm and Cyprus-Paphos. Limnos, Lesvos and Crete populations showed significant departure from HWE due to heterozygote deficiency (Table 3, S7). Significant departure from LE was found only in the Lesvos population (S8). We did not find any locus departing from HWE and LE in any populations; thus, deviations were likely due to null alleles. Hence, we felt confident in not excluding any loci from our analyses. All F_{ST} values obtained by AMOVA were significant (Table 1). The F_{ST} distances showed significant differentiation among most of populations (Table 2). No significant differences among the I_N values were found (S6).

The Bayesian analysis using only Cypriot populations failed to uncover any structure. The assignment indexes were close to 1/K whether using prior population information or not. The Bayesian analysis with all Greek and Cypriot populations suggested a subdivision into four clusters (Fig. 2): (1) Limnos + Lesvos, (2) Chios, (3) Crete (all together) and (4) Cyprus (all together). All individuals but two (Crete-Farm$_{01}$, Lesvos$_{12}$) were assigned (q_i>0.90) to a cluster, while Lesvos$_{14}$ and Lesvos$_{15}$ grouped with individuals from Chios. When we performed the Bayesian clustering for the mentioned chukars (K=3, for Crete-Farm$_{01}$ and Lesvos$_{12}$; K=2 for Lesvos$_{14–15}$), none could be assigned to any group with a P(K) value near 1/K. When we analysed the Greek chukars (n=14) with eastern-type mtDNA lineage (Fig. 1, S4), all but one matched with their island of origin (Lesvos$_{12}$, not assigned).

The FCA of individual STR genotypes accounted for 74.2% of the total variability and showed the same groups inferred by Bayesian clustering (Fig. 3). Gene flow (N_em) was: Paphos-Larnaka=6.1; Paphos-Farm=2.5; Paphos-Karpasia=0.9; Farm-Karpasia=0.8; Larnaka-Farm=4.3; Larnaka-Karpasia=0.8; others were null.

Discussion

The genetic investigation of the chukar island populations of Limnos, Lesvos, Chios, Crete and Cyprus did not disclose any ESUs, even considering different definitions

Table 1 Hierarchical AMOVAs for both mtDNA and STR data

Cyprus: all Cypriot populations by their own; Crete: all Cretan populations by their own; Cyprus + Greece: all Cypriot and Greek populations; All: all 26 populations (Electronic Supplementary Material S1). The φ_{ST} and F_{ST} values are reported together with the relative statistical significance (P)

	Populations (n)	Variability among populations (%)		Variability within populations (%)		φ_{ST}	F_{ST}	P value	
		mtDNA	STR	mtDNA	STR	mtDNA	STR	mtDNA	STR
Cyprus	4	10.61	7.19	89.39	92.81	0.11	0.072	0.0015	<0.001
Crete	2	37.03	16.28	62.97	83.72	0.37	0.163	0.0084	<0.001
Cyprus+ Greece	9	43.44	21.36	56.46	78.64	0.44	0.214	<0.001	<0.001
All	26	54.67	–	45.33	–	0.55	–	<0.001	–

Table 2 φ_{ST} and F_{ST} pairwise distance values among all Greek and Cypriot populations

φ_{ST} mtDNA F_{ST} STR	Chios	Limnos	Lesvos	Crete	Crete-Farm	Cyprus-Paphos	Cyprus-Farm	Cyprus-Larnaka	Cyprus-Karpasia
Chios	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–
Limnos	0.55	–	–	–	–	–	–	–	–
	0.30	–	–	–	–	–	–	–	–
Lesvos	0.43	0.10 (0.028)	–	–	–	–	–	–	–
	0.24	0.06 (0.001)	–	–	–	–	–	–	–
Crete	0.70	0.65	0.65	–	–	–	–	–	–
	0.27	0.25	0.23	–	–	–	–	–	–
Crete-Farm	–	–	–	–	–	–	–	–	–
	0.19	0.34	0.25	0.16	–	–	–	–	–
Cyprus-Paphos	0.63	0.58	0.51	0.72	–	–	–	–	–
	0.27	0.27	0.21	0.25	0.19	–	–	–	–
Cyprus-Farm	0.64	0.59	0.52	0.73	–	0.09 (0.051)	–	–	–
	0.30	0.31	0.24	0.28	0.23	0.00 (0.393)	–	–	–
Cyprus-Larnaka	0.61	0.56	0.49	0.71	–	0.10 (0.018)	0.00 (0.480)	–	–
	0.28	0.32	0.26	0.29	0.23	0.03 (0.054)	0.03 (0.081)	–	–
Cyprus-Karpasia	0.65	0.62	0.56	0.74	–	0.14 (0.014)	0.19 (0.002)	0.10 (0.037)	–
	0.24	0.32	0.26	0.27	0.24	0.12	0.13	0.10	–

For each population, mtDNA values are reported on top of STR values (*P* values between brackets, each pair: unless specifically indicated, all values are <0.001). All samples whose mtDNA lineage belonged to clade B (Fig. 1) were excluded. It was necessary to join Crete and Crete-Farm mtDNA datasets; only STR values are indicated for Crete-Farm on its own

(Fraser and Bernatchez 2001). The majority of chukars clustered into the same mtDNA group, which was differentiated only with respect to populations from East Asia and USA (Fig. 1, S4). Nevertheless, we proved the existence of genetically, and for Cyprus also demographically, well-diverging populations (Table 2). We suggest that they should be treated as distinct MUs.

Genetic structure: mtDNA data

The φ_{ST} distances marked out the following diverging groups: Limnos, Lesvos, Chios, Crete, Crete-Farm, Cyprus-Paphos-Larnaka-Farm and Cyprus-Karpasia (Table 2). Paphos and Larnaka were differentiated, yet both did not diverge from Cyprus-Farm. We suggest that they should be treated as a single group to avoid over-splitting (Allendorf and Luikart 2007).

Literature reports that introductions of chukar from East Asia into USA were carried out beginning in late 1800 (True 1937; Cottam et al. 1940; Christensen 1970). In our study, all the US chukars hold eastern-type haplotype, a result that strengthened the evidence provided by Barbanera et al. (2009a) for the Asian origin of the US chukar population. Moreover, 16 specimens from Greece and Turkey showed eastern-type haplotypes (Fig. 1, S4); thus, they were considered as birds of East Asia origin. In the late 1960s, chukars were imported from UK to Greece for hunting (e.g., to Spetsopoula Island). These birds were likely of Asian origin, as chukars colonising the UK have been previously imported from Italy (Potts 1988; Barbanera et al. 2007). It is allegedly assumed that the Greek Forest Service employed these chukars as breeders (C. Sokos personal communication 2008). However, chukars with mtDNA lineage not corresponding to their phenotype were not found in our samples. It is known that *A. rufa* and *A. graeca* partridges have been introduced into Aegean islands (Papaevangelou et al. 2001) and that these species can hybridise with chukar. The genetic integrity of chukars from Limnos and Cyprus was even more relevant when geographically neighbouring populations were considered. In fact, it is known that red-legged and rock partridge have been introduced into Lebanon (Third National Biodiversity Report to the Convention on Biological Diversity, 2005: http://biodiversity.moe.gov.lb). As a result, wild Lebanese chukars with *A. rufa*-introgressed mtDNA have been disclosed (F. Barbanera unpublished data). Furthermore, two chukars from South Turkey clustered into the eastern clade (clade B: Fig. 1).

Genetic structure: STR data

The F_{ST} distances computed for Greek and Cypriot populations using STR genotypes were in very good

Table 3 mtDNA haplotype diversity (h), mean number of pairwise differences (k) and nucleotide diversity (π, %) values computed for all chukar populations (±SD, standard deviation) together with sample size and number of polymorphic sites (specimens holding clade B haplotypes were excluded; cf., Fig. 1)

Population	mtDNA					STR								
	n	*ps*	*h*±SD	*k*±SD	π±SD,%	*n*	*n*A	*n*a	Ar	I_N±SD	H_O	H_E	*P* value	χ^2 (*df*)
Limnos	22	8	0.76±0.06	1.66±1.01	0.07±0.05	22	3.3	0.4	2.15	0.37±0.07	0.332	0.358	0.003[a]	33 (14)
Lesvos	19	8	0.79±0.06	2.09±1.22	0.09±0.06	20	4.0	0.1	2.42	0.42±0.07	0.321	0.409	<0.001[a]	71 (16)
Chios	18	10	0.88±0.04	2.61±1.46	0.12±0.07	21	5.3	0.5	3.17	0.62±0.06	0.523	0.604	0.009	32 (16)
Crete	22	15	0.86±0.05	2.65±1.47	0.12±0.07	27	5.1	1.0	2.83	0.54±0.06	0.515	0.527	0.002[a]	37 (16)
Crete-Farm	–	–	–	–	–	5	3.1	0.1	2.97	0.59±0.14	0.587	0.523	0.767	12 (16)
Cyprus-Paphos	12	8	0.88±0.07	2.80±1.59	0.12±0.08	12	2.9	0.0	2.30	0.42±0.08	0.418	0.404	0.166	19 (14)
Cyprus-Larnaka	12	13	0.92±0.05	2.90±1.64	0.13±0.08	12	3.3	0.3	2.40	0.42±0.09	0.386	0.398	0.468	14 (14)
Cyprus-Farm	12	10	0.89±0.08	1.94±1.18	0.09±0.06	12	2.5	0.0	2.09	0.36±0.08	0.342	0.348	0.560	13 (14)
Cyprus-Karpasia	12	7	0.92±0.04	2.54±1.47	0.11±0.07	12	3.5	0.4	2.63	0.46±0.07	0.428	0.439	0.009	27 (12)

STR variability for each population: *n* sample size, *n*A number of alleles per locus, *n*a number of unique alleles, *Ar* allelic richness, I_n Nei's index with SD, H_O observed heterozygosity, H_E expected heterozygosity, *P* probability value for HWE test, χ^2 test with relative degrees of freedom (*df*; Fisher global test, all loci)

[a] Significant departure from HWE after application of the Bonferroni correction (α=0.05, $\alpha' = \alpha/8 = 0.006$)

agreement with those provided by mtDNA markers (Table 2). Although Bayesian clustering marked out four groups (Fig. 2), when geographically a priori defined populations correspond closely to detected genetic populations, testing predefined groups for differences in haplotype frequency can provide more powerful and reliable insights into population differentiation than applying STRUCTURE (Pritchard et al. 2007). Given that gene flow among chukar populations of the government-controlled area of Cyprus and the Karpasia population (Turkish-occupied area) was lower than 0.1% of the annual number of harvested animals, we were sure the gene flow was much lower than 10% of the populations' N_e. Overall, significant genetic structure was found (Table 2) and the Karpasia population represented a single MU (Palsbøll et al. 2007), a new result with respect to those reported by Guerrini et al. (2007).

The mtDNA markers disclosed 14 Greek chukars holding maternal lineage of Asian origin (Fig. 1, S4). Nonetheless, when analysed for STRs, all but one clustered with their population of origin as opposed to making out a new group or pooling together with un-assigned individuals. The easiest explanation was that mtDNA genetics is determined by an effective population size that is one fourth as large as that of nuclear genes (Barbanera et al. 2009b). Although biparental STR loci have a higher mutation rate than maternal mtDNA genes, assuming a constant mutation rate, they would need many thousands of generations to accumulate the same amount of difference in isolated populations to provide insight into the exotic origin of Mediterranean chukars (Whitlock and McCauley 1999). Such a period of time would be much longer than the estimated age of chukar in the Mediterranean (Masseti 1997).

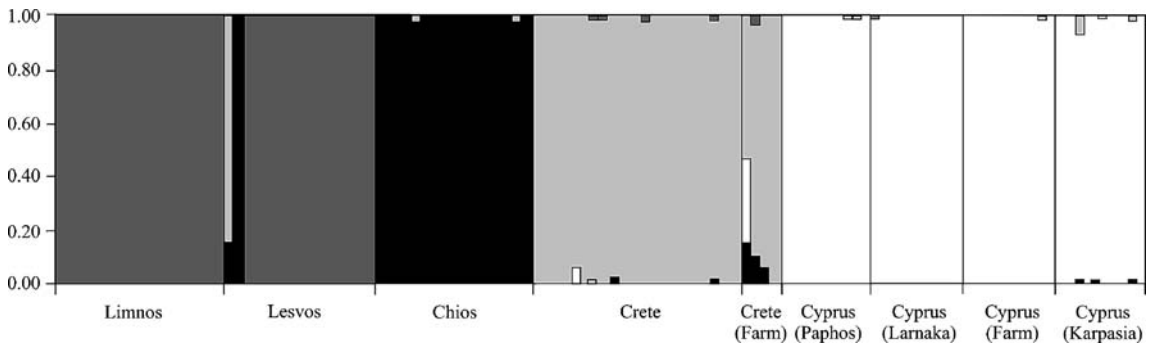


Fig. 2 Bayesian admixture analysis of chukar genotypes computed by structure with K=4. Each individual is represented as a *vertical bar* partitioned in K segments, whose length represents the assignment probability to the Kth cluster. Specimens are grouped according to their population of origin

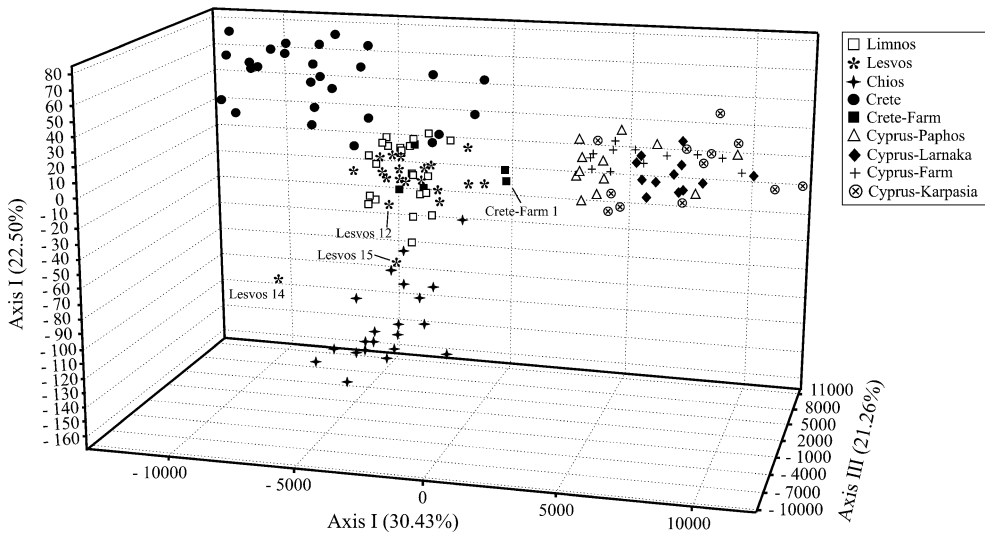


Fig. 3 The factorial correspondence analysis of the individual STR genotypes. Specimens that are not clearly assigned by the Bayesian analysis (Fig. 2) are indicated. Two Lesbian specimens ($Lesvos_{14–15}$) were closer to Chios than to the Lesvos population

Conservation management guidelines

Both mtDNA and STR markers concurrently marked out genetic structure in the studied chukar populations. We suggest six MUs: Limnos, Lesvos, Chios, Crete, Cyprus-Paphos-Larnaka and Cyprus-Karpasia. In the context of rapid environmental changes that the Mediterranean has been experiencing in the last decade, it seems worthy to maintain options for future adaptation. Our conservation strategy aims to preserve locally differentiated genetic resources that might result into ecologically and behaviourally diverging populations (ESUs).

The chukar populations from Limnos and Cyprus are genetically intact and well differentiated, and modern-day import of chukar stocks can be excluded (C. Sokos and P. Panayides personal communication 2008). Hence, they are of primary conservation interest. Although it has been reported that translocations among MUs may be advantageous for maintaining genetic variation (e.g., Mills 2006), it must be stressed that Lemnian and Cypriot chukars belong to different subspecies (*A. chukar kleini* and *A. chukar cypriotes*, respectively: Madge and McGowan 2002). Moreover, the extent to which differences in adaptation constrain the viability of populations subject to translocation would need a large study on ecology and behaviour. At this time, we suggest to avoid any translocation between Limnos and Cyprus. In the event of the creation of farmed stocks on Limnos, the use of local breeders would be compulsory.

The presence of chukars of Asian origin on Lesvos (one out of 20), Chios (three out of 21) and Crete (ten out of 32) revealed recent introduction events. Considering that other Aegean islands might host birds of exotic origin, both Greek and Cypriot Institutions should not import from abroad or translocate any chukars among their islands. On the other hand, bird trapping coupled to genetic identification to eradicate non-native genotypes would seem unpractical and highly expensive. Nevertheless, for Lesvos and Chios, we suggest the creation of a genetically controlled stock (50–100 pairs) of wild-caught chukars to be used as source for restocking plans. Owing to the small size of these islands, mating between chukars with native mtDNA and wild conspecifics could erase at least the exotic maternal genes within a relatively low number of generations (Barbanera et al. 2007).

In Crete, not only the sale but also the release in the wild of farmed birds should be blocked. A deeper insight into the total number of non-native birds would be necessary. In the wild, efforts should be made to find out genetically preserved chukars. Their discovery would have conservation merits as the genetic divergence of Cretan and Cypriot populations is in agreement with the morphological evidence that Cretan *A. chukar cypriotes* (formerly *A. chukar scotti*: Madge and McGowan 2002) birds are smaller and darker than Cypriot ones.

In Cyprus, the collection of eggs in the wild for restocking purpose should be carried out according to the pattern of genetic differentiation reported in our study. Given the lack of geographic barriers between the Turkish-occupied part and the region comprised between Paphos and Larnaka (S1), a shared management of the whole chukar population should be pursued. Information about how captive breeding is being undertaken in the occupied territory should be made available. It is well-known that gene flow records need to be taken with extreme caution as many assumptions are involved in their estimate (Whitlock and McCauley 1999). Both mtDNA and STR markers disclosed genetic mixing between Paphos and Larnaka. In the region of Paphos (S1), the release of farmed birds from Stavrouvoni regularly occurs, with some releases occasionally carried out 5 km away from the Larnaka sampling area (P. Panayides personal communication 2008). Given the distance between Paphos and Larnaka (about 150 km), it seems reasonable that the

Stavrouvoni farm worked as a "bridge" between the two populations. Tejedor et al. (2005) reported that hunting pressure might force chukars to move for several kilometres, thus causing genetic mixing among populations. We suggest that restocking plans can explain admixture at least between Paphos and Larnaka populations. Hadjisterkotis (1999) found that survival of radio-marked chukars released in the wild was very low in Cyprus: 44% after 10 days and only 2% after 100 days. Although we agree that majority of released chukars do not enter the wild breeding populations, our results suggest that genetic analysis of captive chukars must be taken before they are used in restocking plans.

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