Conservation genetics and management of the Chukar Partridge *Alectoris chukar* in Cyprus and the Middle East

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The Chukar Partridge *Alectoris chukar* (Phasianidae) is a popular game bird whose range extends from the Balkans to eastern Asia. The Chukar is threatened by human-mediated hybridization either with congeneric species (Red-Legged *A. rufa* and Rock *A. graeca* Partridges) from Europe or exotic conspecifics (from eastern Asia), mainly through introductions. We investigated Chukar populations of the Middle East (Cyprus, Turkey, Lebanon, Israel, Armenia, Georgia, Iran and Turkmenistan: n = 89 specimens) in order to obtain useful genetic information for the management of this species. We sequenced the entire mitochondrial DNA (mtDNA) Control Region using Mediterranean (Greece: n = 27) and eastern Asian (China: n = 18) populations as intraspecific outgroups. The Cypriot Chukars (wild and farmed birds) showed high diversity and only native genotypes; signatures of both demographic and spatial expansion were found. Our dataset suggests that Cyprus holds the most ancient *A. chukar* haplotype of the Middle East. We found *A. rufa* mtDNA lineage in Lebanese Chukars as well as *A. chukar* haplotypes of Chinese origin in Greek and Turkish Chukars. Given the very real risk of genetic pollution, we conclude that present management of game species such as the Chukar cannot avoid anymore the use of molecular tools. We recommend that Chukars must not be translocated from elsewhere to Cyprus.

INTRODUCTION

The distribution range of the most widespread species of *Alectoris* partridge, the Chukar (*A. chukar*, Phasianidae, Plate 1), is claimed to extend from the Balkans to eastern Asia. Several *A. chukar* morphologic subspecies inhabit the Middle East: (1) *A. c. kleini* in north Turkey and Georgia, (2) *A. c. cypriotes* in south Turkey, Cyprus, north Syria, Lebanon, and north Israel, (3) *A. c. sinaica* in south Israel and Jordan, (4) *A. c. kurdestanica* in east Turkey, Transcaucasia, north Iraq and northwest Iran, (5) *A. c. werae* in east Iraq and southwest Iran, (6) *A. c. koroviakovi* in east Iran and (7) *A. c. shestoperovi* in Turkmenistan (Figure 1, Madge & McGowan 2002, Clements 2007). The Chukar is a popular game bird. Hunting pressure, poaching and habitat degradation have warranted its inclusion in the Species

Plate 1. Chukar Partridge *Alectoris chukar* photographed in the wild, Cyprus. © *P Panayides*

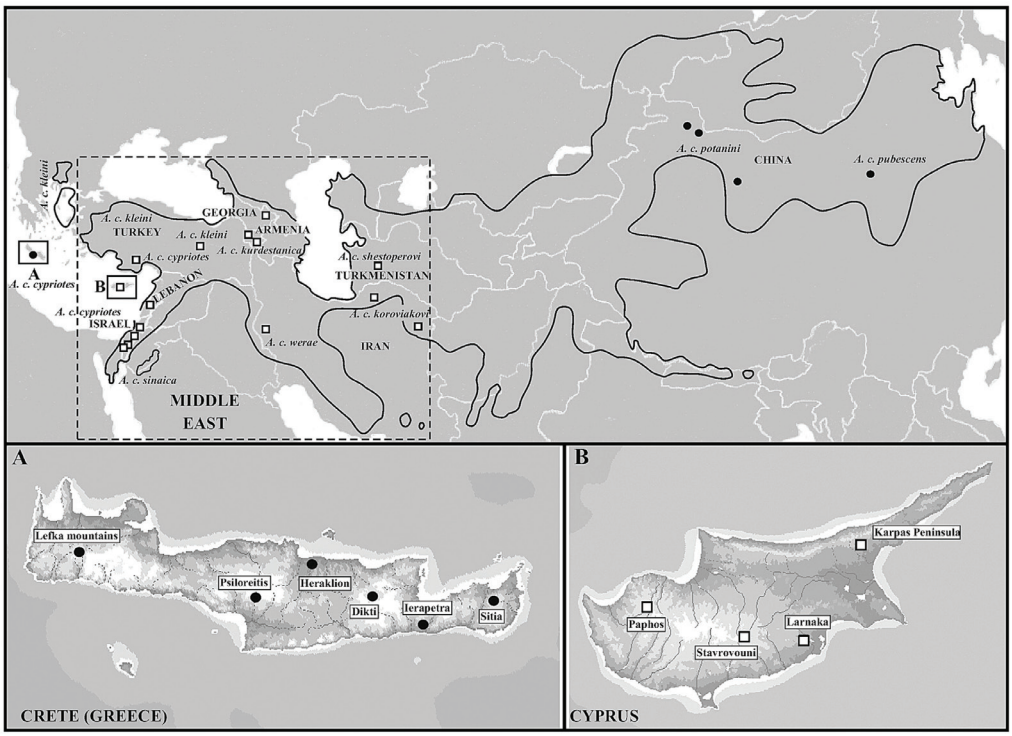

Figure 1. Distribution of the Chukar Partridge *Alectoris chukar* (black line) with the subspecies investigated in this study. The Middle East region as understood in this paper is indicated by the rectangular dotted line (white squares indicate sampling sites). In China, black circles show sampling sites. A: sampling sites in Crete; B: sampling sites in Cyprus.

of European Conservation Concern (BirdLife International 2004) yet it is listed as Least Concern by IUCN (BirdLife International 2010).

In 2004 the Cypriot government promoted a project for the conservation of the Chukar. The strong pressure on wildlife resources over the last 20 years due to human overcrowding, rapid land-use change and economic development have largely contributed to habitat loss and/or degradation. Resort building sprawl, construction of a dense road network, change of traditional agriculture with increased mechanization and pesticide use as well as hunting pressure represented other important threatening factors to the Chukar as well as to the rest of the wildlife resources of Cyprus (Panayides 2005). Since the 1970s the number of suitable hunting territories has greatly decreased, the land-use pressures and potential limiting factors have increased whereas the number of hunters basically remained unchanged (*c*50 000 in the government controlled area). In order to face these increasing challenges, the Game Fund Department, Ministry of the Interior, started in 1990 a release program of Chukars in Cyprus. Releases started from 18 000 (1990), reached a maximum of 270 000 in 1999 (Kassinis 2001), and then levelled to about 120 000–130 000 birds/year (2005–2010).

The release of captive-bred individuals without genetic control represents a potential major pathway of introduction of exotic genotypes, a threat that may lead to genetic homogenization by gradually disrupting adaptations of local biotas through hybridization (McKinney & Lockwood 1999, Olden *et al* 2004, Romagosa *et al* 2009, Barbanera *et al* 2010). Although in the wild about 10% of avian species do hybridize (McCarthy 2006), among the game birds human-mediated hybridization can be much more common. This is the

case in the order Galliformes (21.5% of hybridizing species: Grant & Grant 1992) and especially of the genus *Alectoris*, where anthropogenic hybridization occurs between the Chukar and Rock Partridge *A. graeca* (in Greece and Turkey: Barilani *et al* 2007, BirdLife International 2010) or Red-Legged Partridge *A. rufa* (in southwest Europe: Barbanera *et al* 2007, 2010). Natural hybridization between the Chukar and the Rusty-necklaced Partridge *A. magna* occurs in China (*eg* Huang *et al* 2009). In general, a few morphological pointers can be used to identify first-generation *Alectoris* hybrids, but strong uncertainty remains as backcross hybrids are easily missed (McCarthy 2006). As a result, when farmed birds are released to reinforce wild populations the risk of 'pollution' is strong in the absence of genetic controls.

Cyprus still harbours a healthy Chukar population. Genetic investigation is fundamental to bring adaptive conservation management of a given species into line with kinship of its populations. This paper aims to provide genetic information (population structure and demographic inferences) to aid management of the Chukar in Cyprus and the Middle East using data of Barbanera *et al* (2009b). We analysed Cypriot and Asian mainland populations by sequencing the entire mitochondrial DNA (mtDNA) Control Region and using both Mediterranean and eastern Asian Chukars as intraspecific outgroups.

METHODS

Biological sampling

One hundred and thirty-four Chukar samples were collected (Table 1). In Cyprus, liver samples from wild birds were obtained from hunted specimens in the areas of Larnaka and the Karpas peninsula, whereas feathers were taken from Chukars kept in the Stavrovouni government farm (Figure 1B). We obtained Chukar samples collected on the ground (feathers) or obtained from trapped birds (blood) from most of the Middle Eastern countries. Finally, we used both Mediterranean (*A. c. cypriotes* Crete) and east Asian Chukars (*A. c. potanini* and *A. c. pubescens* China) as intraspecific outgroups (Table 1, Figure 1). The Natural History Museum of Crete and the Hunting Greek Confederation provided the Cretan samples, while the Xinjiang Institute of Ecology and Geography (Urumqi) and the School of Life Sciences (Lanzhou) those from China.

Table 1. Geographical location, number of samples and type of tissue used for Chukar Partridges investigated in this study.

Country	Region/locality	Samples (n)	Tissue
MIDDLE EAST			
Cyprus	Paphos	12	Liver
Cyprus	Larnaka	12	Liver
Cyprus	Stavrovouni farm	12	Feather
Cyprus	Karpasia	12	Liver
Turkey	Adiyaman	1	Feather
Turkey	Mersin	2	Feather
Lebanon	Aammiq	6	Feather
Israel	Several localities	7	Liver
Georgia	Kahetia	2	Liver
Armenia	Garni	2	Blood
Armenia	Yeghegnadzor	4	Liver
Iran	Ilam	4	Feather
Iran	North Khorasan	4	Feather
Iran	Razavi Khorasan	4	Feather
Turkmenistan	Garrygala	5	Blood
Sub-total		*89*	
EUROPE			
Greece	Crete	27	Liver
Sub-total		*27*	
ASIA			
China	Aibi	2	Feather
China	Qi-Lian	2	Blood
China	Baytag	5	Feather
China	Zheng	9	Feather
Sub-total		*18*	
TOTAL		**134**	

DNA extraction, amplification and sequencing

DNA was extracted from liver and blood using the PUREGENE CORE KIT-A (Qiagen) following the manufacturer's instructions, and from feathers as in Barbanera *et al* (2005). We amplified the entire mtDNA

Control Region gene (CR) as in Barbanera *et al* (2005). We purified and directly sequenced the PCR products on both DNA strands as in Barbanera *et al* (2009a).

Genetic diversity

We aligned 134 CR sequences using CLUSTALW (v. 1.81: Thompson *et al* 1994). We employed MEGA (v. 4.1: Kumar *et al* 2008) to check for neutral evolution of the entire mtDNA ingroup using Tajima's *D* (Tajima 1989). The partition of genetic diversity was investigated by AMOVA (analysis of molecular variance, with two hierarchical levels: among populations and among individuals within populations) with ARLEQUIN (v. 3.01: Excoffier *et al* 2005) using the ϕ_{ST} analogous of Wright's (1951) *F* statistics (1000 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 also used to calculate the nucleotide diversity (π), the mean number of pairwise differences (*k*) and the haplotype diversity (*h*). We created a network using the Median Joining method (Bandelt *et al* 1999) with NETWORK (v. 4.5.1.0, © 2004–2009 Fluxus Technology).

Demographic inferences

We pooled together all wild and farmed Cypriot specimens (n = 48) in order to get an approximate estimate of the demographic history of the Chukar on the island. Computations were carried out using DNASP (v. 5.1: Librado & Rozas 2009). Ramos-Onsins & Rosas (2002) demonstrated that *Fs* statistics of Fu (1997) have the greatest power to detect population expansion (*versus* R_2 statistics or Tajima's *D*) especially when sample sizes are large (~ 50). *Fs* statistics are based on the probability of having a number of haplotypes greater or equal to the observed number of samples drawn from a constant-sized population. We tested the significance of the *Fs* statistics (H_0: population constant size) by examining the null distribution of 5000 coalescent simulations using DNASP as in Ramirez-Soriano *et al* (2008). Only highly significant and negative *Fs* values were retained as evidence of population expansion (Pilkington *et al* 2008). The mismatch distributions (MD, H_0: sudden expansion model) of mtDNA pairwise distances were examined using ARLEQUIN to get insight into both pure demographic and spatial population expansion. In the first case, the more ragged the shape of the distribution, the closest the population to a stationary model of constant size over a long period. We computed Harpending's raggedness index (r, Harpending *et al* 1993). This index takes larger values for multimodal distributions commonly found in a stationary population than for unimodal and smoother distributions typical of expanding populations. While MD describes the pairwise differences between haplotypes, the raggedness index indicates the variation along the curve. MD tests observed parameters of the expansion to perform coalescent simulations and to create new estimates of the same parameters. We tested departure from a model of expansion by summing the squared differences (SSD) between observed and estimated MD (Excoffier 2004). In ARLEQUIN the parameters of a spatial expansion are estimated using the same least-square method as in the estimation of a pure demographic expansion (see ARLEQUIN v. 3.01, user manual, pp 93–96). The use of 'SSD' statistics herein refers to the MD test for pure demographic expansion, whereas that of 'SSD*' statistics refers to the MD test for spatial expansion.

RESULTS AND DISCUSSION

Genetic diversity

All specimens showed the expected *A. chukar* mtDNA lineage, with the exception of three Chukars from Lebanon holding the *A. rufa* mtDNA lineage. This result did not come as a surprise as the introduction for hunting of *A. rufa* and *A. graeca* in Lebanon is well-known

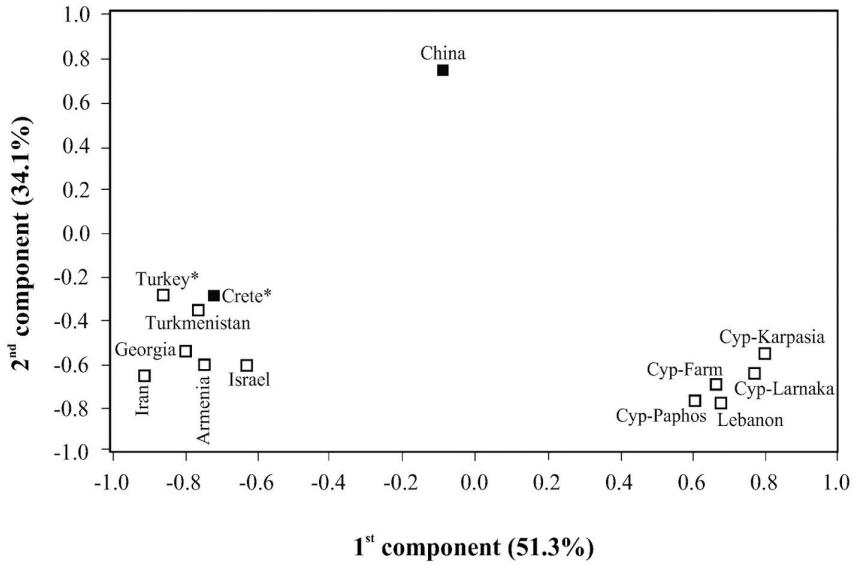

Figure 2. The PCA plot with the pairwise ϕ_{ST} distances (Table 3) calculated using the sequences of all Chukar Partridge *Alectoris chukar* populations. * allochthonous *A. rufa* sequences are excluded from Lebanon as well as those of Chinese origin from Crete and Turkey. White squares are used for Middle East populations while black squares for outgroups. Cyp, Cyprus.

(Madge & McGowan 2002, Third National Biodiversity Report to the Convention on Biological Diversity 2005, http://biodiversity.moe.gov.lb).

The alignment of 131 (*ie* 134 − 3) *A. chukar* CR sequences defined a set of 1156 characters and fitted to a neutral model of evolution (Tajima's *D* for the entire ingroup = − 0.330, *P* = 0.39). When the mtDNA diversity among all populations (Table 2**)** was analysed, AMOVA indicated that 56.0% of the total genetic variability was distributed among populations and 44.0% among individuals within populations (ϕ_{ST} = 0.56, *P* < 0.001). When the ϕ_{ST} distance values (Table 3**)** were plotted on a PCA (Figure 2), the two components explained 82.1% of the total diversity. The Cypriot and Lebanese populations clustered in the lower right corner of the plot, whereas all the remaining Middle East Chukars grouped in the opposite one, the Cretan population lay in between (but see below and Figure 4). All aforementioned populations were well separated with respect to the Chinese subspecies. For instance, the ϕ_{ST} distance value between Cyprus (or Lebanon) and the remaining Middle East populations (also Crete)

Table 2. MtDNA haplotype diversity (*h*), mean number of pairwise differences (*k*) and nucleotide diversity (π, %) values computed for all Chukar Partridge populations (± SD, standard deviation) together with sample size and number of haplotypes (na). * five Chukars of Asian origin were excluded (Figure 4), ** two Chukars of Asian origin were excluded, *** three Chukars with *A. rufa* mtDNA lineage were excluded. Cyp, Cyprus; Farm, Stavrovouni farm.

Population	n	na	*h* ± SD	*k* ± SD	π ± SD (%)
Crete*	22	6	0.76 ± 0.06	3.40 ± 1.81	0.29 ± 0.17
Cyp-Larnaka	12	7	0.88 ± 0.07	1.44 ± 0.94	0.12 ± 0.09
Cyp-Paphos	12	6	0.84 ± 0.07	2.14 ± 1.28	0.18 ± 0.12
Cyp-Karpasia	12	3	0.67 ± 0.09	0.80 ± 0.60	0.07 ± 0.06
Cyp-Farm	12	4	0.56 ± 0.15	1.10 ± 0.78	0.10 ± 0.07
Turkey**	1	1	-	-	-
Lebanon***	3	3	1.00 ± 0.27	1.34 ± 1.10	0.11 ± 0.10
Israel	7	5	0.86 ± 0.13	3.72 ± 2.14	0.32 ± 0.21
Armenia	6	4	0.87 ± 0.13	3.01 ± 1.82	0.26 ± 0.18
Georgia	2	2	1.00 ± 0.50	4.01 ± 3.17	0.35 ± 0.38
Iran	12	5	0.83 ± 0.07	2.98 ± 1.67	0.27 ±0.16
Turkmenistan	5	3	0.70 ± 0.21	1.60 ± 1.13	0.14 ± 0.11
China	18	7	0.90 ± 0.31	1.86 ± 1.11	0.16 ± 0.11

Table 3. For each Chukar Partridge population pair the ϕ_{ST} pairwise distance values among all populations are reported under the diagonal, while the *P* values are reported above it. Allochthonous sequences of Chinese origin in Cretan and Turkish populations were excluded. Cyp-L, Cyprus-Larnaka; Cyp-F, Cyprus-Farm; Cyp-P, Cyprus-Paphos; Cyp-K, Cyprus-Karpasia; Turkm., Turkmenistan.

ϕ_{ST}	Crete	China	Cyp-L	Cyp-F	Cyp-P	Cyp-K	Turkey	Iran	Lebanon	Israel	Armenia	Turkm.	Georgia
Crete	*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0	0	> 0.99	0.03
China	0.79	*	< 0.001	< 0.001	< 0.001	< 0.001	> 0.99	< 0.001	0.001	0	0	0	0.01
Cyp-L	0.65	0.84	*	0.79	0.05	0.32	> 0.99	< 0.001	0.28	0	< 0.001	0	0.01
Cyp-F	0.65	0.84	0	*	0.06	0.02	> 0.99	< 0.001	0.16	0	0	0	0.02
Cyp-P	0.64	0.80	0.09	0.10	*	0.04	> 0.99	< 0.001	0.09	0	< 0.001	< 0.001	0.01
Cyp-K	0.67	0.86	0.02	0.13	0.14	*	> 0.99	< 0.001	0.08	0	0	< 0.001	0.01
Turkey	0.50	0.73	0.62	0.66	0.52	0.77	*	0.15	0.25	0.25	0.12	0.17	> 0.99
Iran	0.47	0.76	0.44	0.45	0.43	0.51	0.11	*	0.004	0.003	0.02	0.04	0.49
Lebanon	0.60	0.83	0.06	0.11	0.13	0.22	0.50	0.38	*	0.02	0.06	0.02	0.10
Israel	0.56	0.71	0.43	0.45	0.32	0.51	0.14	0.25	0.30	*	0.003	0.005	0.22
Armenia	0.51	0.78	0.42	0.46	0.42	0.50	0.12	0.18	0.27	0.24	*	0.11	0.61
Turkm.	0.52	0.81	0.62	0.65	0.54	0.72	0.58	0.20	0.58	0.30	0.15	*	0.33
Georgia	0.49	0.81	0.48	0.55	0.43	0.63	0	0.03	0.31	0.13	0	0.17	*

were all highly significant ($P < 0.001$, not shown), the genetic distance (Tamura and Nei 1993 algorithm; data not shown) ranging around 0.30% (versus Chinese Chukar, around 0.80%). This average value does not support the existence of several *A. chukar* subspecies in the Middle East (see Introduction).

Haplotype diversity, nucleotide diversity and mean number of pairwise differences of wild Cypriot populations of Paphos and Larnaka did not significantly differ from each other as well as versus populations with comparable sample size (*eg* Crete, Iran and China). Larnaka and Paphos populations showed higher genetic diversity than the Karpasia peninsula population and the stock kept at the Stavrovouni farm (Table 2). However, an in-depth investigation carried out using microsatellite DNA markers proved that wild populations of Paphos-Larnaka and Karpasia peninsula should be treated as two separated management units (*sensu* Palsbøll *et al* 2007, see Barbanera *et al* 2009b).

The network in Figure 3 shows that all Middle East populations were connected to each other and lay between the Cretan and Chinese groups. We found 45 haplotypes among the 131 sequences (GenBank accession codes: Barbanera *et al* 2009b). The most frequent was H30, which was shown almost only (95%) by Cypriot Chukars (5% by Lebanese Chukars). Of these former, 42% were captive-bred birds kept in the Stavrovouni farm. Although the sample sizes of investigated populations were not all comparable (Table 1), the coalescent theory (Crandall & Templeton 1993) predicts that the most frequent and widespread haplotype is the most ancient one. Our dataset suggests that Cyprus holds the most ancient *A. chukar* haplotype of the Middle East, although this result needs to be confirmed by the investigation of a larger sample size for each continental Asian population. Furthermore, the star-like topology (Figure 3) showing many haplotypes (H27 to H29, H31 to H38: the very large majority from Cyprus) stemming from the most common one (H30), suggests population expansion of the Cypriot Chukar (see below, also Randi *et al* 2003). The network also indicated that five Cretan and two Turkish specimens shared haplotypes with Chinese Chukars (H1 and H3, respectively, Figure 3). In this regard, Barbanera *et al* (2009a) proved the existence of a human-mediated *A. chukar* gene flow from eastern Asia

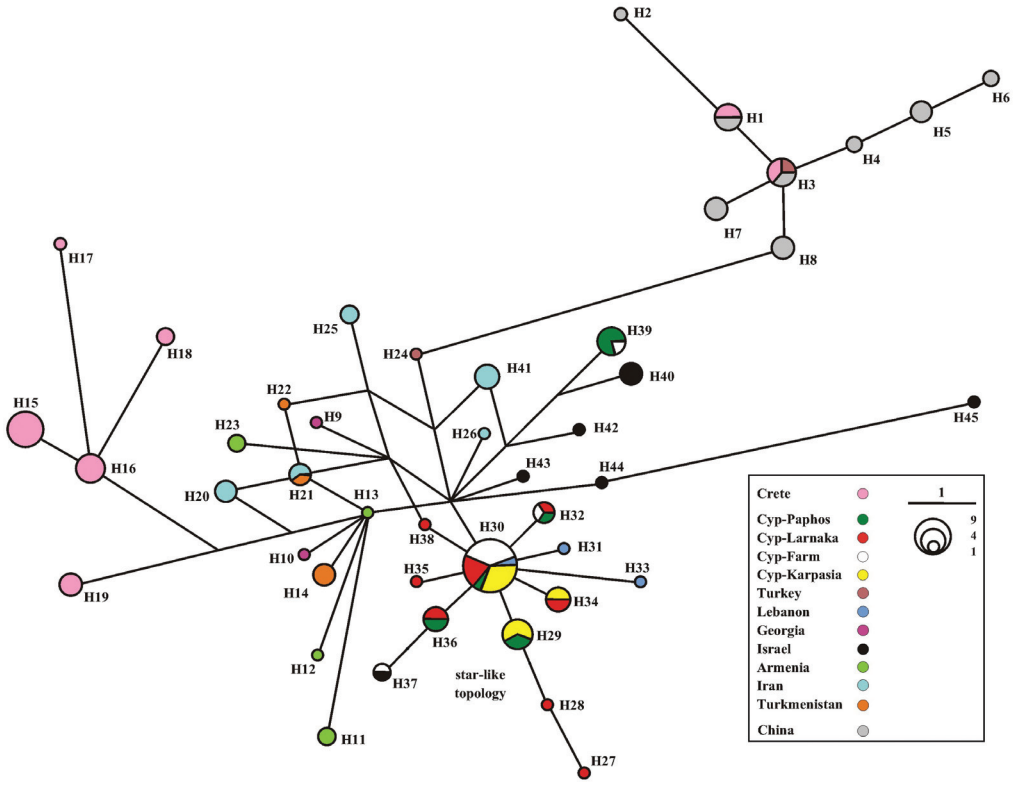

Figure 3. The mtDNA network computed for all 45 Chukar Partridge *Alectoris chukar* haplotypes (H). Each population is marked by its own colour; the length of the bar refers to a single mutational change, while the diameter of each circle is proportional to the number of specimens with a given haplotype.

(from China, see also Martinez-Fresno *et al* 2008) to the Mediterranean. In contrast to the *A. rufa* mtDNA-introgressed Lebanese Chukars, the Greek and Turkish Chukars holding genes of Chinese origin showed *A. chukar* intraspecific pollution. However, in both cases, genetic controls could have prevented the mixing of Chukars.

Demographic inferences

We grouped all Cypriot Chukar sequences in order to get useful yet approximate insight into the historical demography of the species on the island (*eg* see Zink *et al* 2006). We found a highly significant and negative *Fs* value ($Fs = -19.60$, $P < 0.001$), which provides strong evidence for Cypriot population expansion. In Figure 4 the mismatch distribution (MD) of the pairwise mtDNA differences is given. The L-shaped curve with a small number of differentiating mutations among individuals suggests recent population expansion. The relative statistics revealed that models of demographic (SSD) and spatial (SSD*) expansion could be not rejected ($P = 0.29$ and $P = 0.43$ respectively). Moreover, the non-significant value of the raggedness index ($r = 0.06$, $P = 0.28$) indicates that the fit of our data to a model of population expansion was good. Although the failure to reject the null hypothesis of expansion did not necessarily mean that the Cypriot population had undergone one, the curve in Figure 4 does not support any alternative hypothesis of stability, and fitted to the star-like topology of Figure 3. Thus, it seems very likely that the Cypriot Chukar population has recently experienced both demographic and spatial expansion.

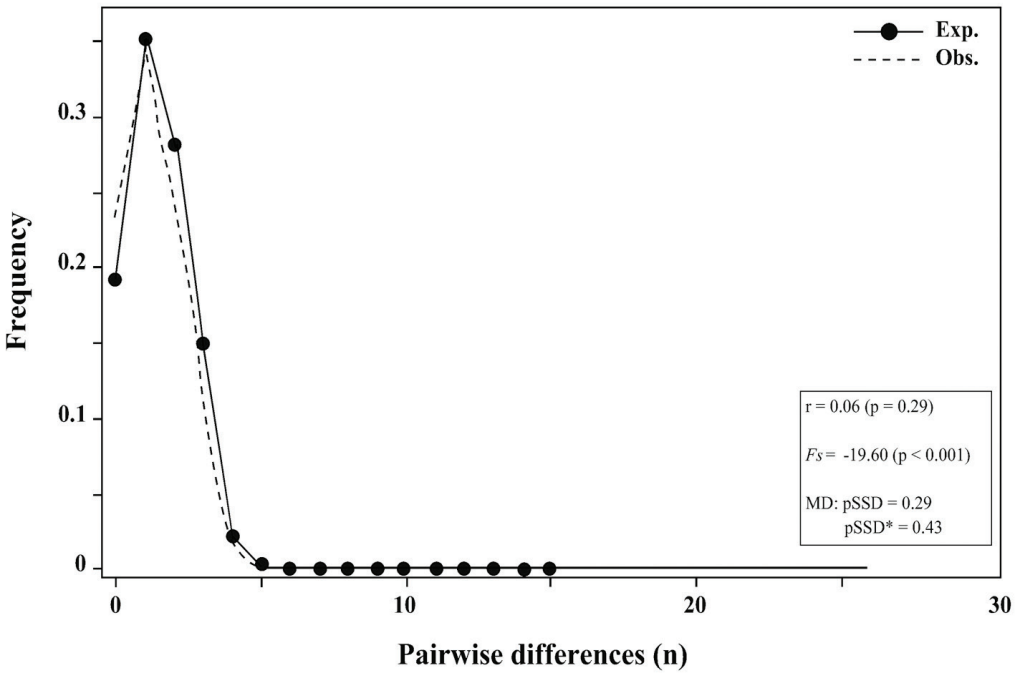

Figure 4. Distribution of the pairwise differences (observed: dotted; expected: line) calculated for the entire Cypriot population of Chukar Partridges *Alectoris chukar* sampled (n = 48, Table 1). Harpending's raggedness index (r) and *Fs* values are given with their significance; the *P* values of the SSD and SSD* statistics from the mismatch distribution (MD) are also reported under a model of sudden demographic and spatial expansion, respectively (see text for details).

Management implications

Increasing pressures such as habitat loss, rapid land use changes and severe hunting pressure represent the most relevant threats to the conservation of *Alectoris chukar*. Management of the Chukar including the release of farmed birds must safeguard the indigenous populations, as the preservation of native genotypes is crucial. For instance, Potts (1989) reported that the release of *A. rufa* x *A. chukar* hybrids in the UK has decreased the reproductive rate of local *A. rufa* populations because hybrids have lower fitness in the wild than pure partridges. In Cyprus, careful management including genetic screening has warranted purity and diversity of the local Chukars. Barbanera *et al* (2007), using nuclear DNA markers, showed that the same Cypriot populations of that paper did not include any *A. rufa* x *A. chukar* hybrids. It follows that the import of Chukars from Lebanon to Cyprus must be strictly prohibited because of the genetic pollution affecting the former population. Preventive measures against potential illegal introductions should be also enforced. In order to comply with the Chukar intraspecific structure (Figure 2), it would be also highly recommendable not to import Chukars to Cyprus from any other Middle Eastern country (*eg* Turkey) or Greece. On the other hand, Cypriot Chukars could be suitable founders for an *ex-situ* programme to improve the conservation status of the Lebanese *A. chukar* (Figures 2 & 4).

We realize that use of molecular techniques can be costly. Nonetheless, it would be in the best interest not only of governmental institutions but also of hunters and farmers. The Cypriot Game Fund Department pursued the adaptive conservation management of the Chukar Partridge bringing the exchange of captive stocks into line with knowledge of

their genetic kinship with wild populations. We encourage all countries of the Middle East to apply similar molecular screening.

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