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Current status of the Sardinian partridge (*Alectoris barbara*) assessed by molecular markers

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Abstract Four *Alectoris* species inhabit the Mediterranean area, where they represent important gamebirds subject to human manipulations. The Sardinian partridge is peculiar in Europe, in that it belongs to the African species *Alectoris barbara*. Nevertheless, no comprehensive study has as yet investigated its genetic status as regards both the extant levels of genetic diversity and the possible contamination due to introgressive hybridization with other Mediterranean species. For the purposes of this study, we analyzed 65 samples of Sardinian partridges, 40 of which came from the wild population and 25 from captive stocks. No one of them showed a mtDNA polymerase chain reaction restriction fragment length polymorphism haplotype assigned to another species than *A. barbara*, thus, ruling out a possible introgression in the maternal line. In addition, we compared these samples with 94 partridges from other circum-Mediterranean populations using a set of eight chicken

(*Gallus gallus*) microsatellites. A low level of genetic variation was observed in the Sardinian population ($H_E=0.310$; $k_{AR}=2.69$), comparable only to that observed in the Sicilian rock partridge (*A. graeca*). The comparison with the Tunisian population showed that its present genetic composition is consistent with a historical introduction from North Africa, showing possible effects of a post-introductory genetic drift. Bayesian tests assigned all but one individuals with >90% probability to *A. barbara*, thus, providing evidence that no or only a few exotic *Alectoris* genes have introgressed into Sardinian partridges.

Keywords Sardinia · Barbary partridge · Genetic variability · Introgressive hybridization · Microsatellites

Introduction

Island populations are a major concern in conservation genetics. This is because of the important genetic implications (e.g., genetic drift, bottlenecks, inbreeding) that can affect the status of a long-term isolated population. Founder effect and genetic drift, associated with low levels of gene flow to and from other populations, can cause a remarkable loss of genetic diversity, compromising the species' adaptability and finally increasing its extinction risk (Allendorf and Luikart 2007). In addition, introgressive hybridization of the local fauna with translocated nonindigenous animals can account for the disruption of locally adapted gene pools, finally causing the genetic extinction of a species or a population (Rhymer and Simberloff 1996; Allendorf et al. 2001).

The terrestrial fauna of most Mediterranean islands has radically changed since the Early Holocene. Native species

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(especially mammals) have, in many cases, been replaced by allochthonous fauna, often as a result of human-mediated introductions, either deliberate or unintentional (Blondel and Vigne 1993). However, these introductions occurred at different prehistorical and historical time points and, to some extent, were followed by local adaptation via genetic differentiation of introduced stocks from their source populations.

Birds belonging to the genus *Alectoris* are a good example of game fauna largely managed by humans and intentionally introduced on many Mediterranean islands (Johnsgard 1988). In the Mediterranean basin, this genus numbers four species with a basically allopatric distribution, namely: (1) the barbary partridge *Alectoris barbara* in North Africa, (2) the red-legged partridge *A. rufa* in Southwestern Europe from Portugal to Italy, (3) the rock partridge *A. graeca* in Southeastern Europe from the Balkans to Italy, and (4) the chukar partridge *A. chukar* in Eastern Asia (Cramp and Simmons 1980).

Of the four Mediterranean partridges, the barbary partridge is the oldest and the most phylogenetically divergent taxon (Randi 1996; Randi et al. 1998; Kimball et al. 1999). Its speciation is supposed to have occurred at the Miocene–Pliocene boundary, when the closure of the Mediterranean Sea favored the spread of birds adapted to arid and steppe habitats (Randi et al. 1992). Nowadays, the only non-African regions where this species occurs are the Gibraltar peninsula, Sardinia, and the Canary islands (Cramp and Simmons 1980). Regarding the origin of the Sardinian population, two hypotheses were formulated in the past: one proposes that partridges have been introduced by humans at some time during the last 3,000 years (Mocci Demartis 1992), while, according to the other, partridges reached the island by active dispersal from North Africa during the Upper Miocene (Spanò 1975). Palaeontological and zooarcheological records seem to support the former hypothesis and date the introduction at 3,000–2,000 years ago (B. Wilkens, pers. comm.).

Despite its relevancy to the management of the Sardinian population, no genetic investigation has so far been performed to determine its degree of genetic differentiation from North African populations. In addition, the growing interest in rearing captive stocks of this species to satisfy hunters requests, prompts an evaluation of the genetic integrity of the Sardinian partridge population, in particular with respect to the possibility of past hybridizations with other *Alectoris* taxa. In fact, interspecific hybridization within this genus is possible (Watson 1962a, b) and naturally occurs in a few areas of parapatry, such as along the French Alps (between *A. rufa* and *A. graeca*, Bernard-Laurent 1984; Randi and Bernard-Laurent 1999) and in Thrace (between *A. graeca* and *A. chukar*, Dragoev 1974). Furthermore, the occurrence of hybrids is increasingly

found to be a rule in many restocked partridge populations across Europe (Negro et al. 2001; Baratti et al. 2005; Barilani et al. 2007a, b; Martínez-Fresno et al. 2008). Noticeably, due to the biogeographical distribution pattern of *Alectoris* partridges in the Mediterranean basin, continental stocks introduced to Sardinia for hunting purposes would quite likely belong to a different species. Therefore, concern has arisen on the risk of introgression of exotic genes into the Sardinian population.

In the present study, we combined polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and microsatellite analyses with the aim to assess the genetic status of the Sardinian partridge population. Multilocus genotyping techniques have already shown to be useful tools for assessing levels of genetic variation and population structure (Randi et al. 2003; Tejedor et al. 2005; Chen et al. 2006; Arruga et al. 2007), as well as resolving interspecific hybridization processes in *Alectoris* taxa (Baratti et al. 2005; Barilani et al. 2007a, b; Tejedor et al. 2007). The microsatellite markers used in these studies had been isolated in the chicken (*Gallus gallus*) and revealed to be polymorphic in species of the genus *Alectoris* (Baratti et al. 2005), as well as in other Galliformes (Baratti et al. 2001).

Goals of our study were: (1) to evaluate levels of genetic variation within the Sardinian stock; (2) to assess the extent of genetic differentiation between the Sardinian population and North African barbary partridges, (3) to check for possible signs of introgressive hybridization with other continental *Alectoris* species.

Materials and methods

Sample collection and DNA isolation

Sixty-five muscle or feather samples were collected in Sardinia from free-living barbary partridges ($n=40$) and from four different captive breeding stocks (Bonassai, $n=6$; Abbasanta, $n=7$; Tertenia, $n=2$; Uta, $n=10$). Sardinian samples are cumulatively coded as AbSar and their location is shown in Fig. 1.

For purposes of comparison, reference samples (blood, muscle, or feathers) were collected from wild *Alectoris* populations across the Mediterranean basin (Fig. 1): barbary partridges *A. barbara* from Tunisia (AbTun, $n=10$), rock partridges *A. graeca* from Sicily (AgSic, $n=10$), Central Apennines (AgApe, $n=20$), and Eastern Italian Alps (AgAlp, $n=10$), chukar partridges *A. chukar* from Israel (AcIsr, $n=17$), and red-legged partridges *A. rufa* from Spain (ArSpa, $n=10$) and from an Italian breeding stock (ArAul, $n=17$). Sampled individuals were classified on the basis of morphological criteria, according to Cramp and

Fig. 1 Locations of samples used in this study. On the *right* are sampling areas and natural ranges of *Alectoris* species in the Mediterranean basin (modified from Cramp and Simmons 1980). On the *left* are sampling sites in Sardinia (wild partridges—black dots,

captive-reared partridges—white dots). Sampled populations are abbreviated as following: *AbSar* Sardinia, *AbTun* Tunisia, *ArSpa* Spain, *ArAul* Aulla (Italy), *AgSic* Sicily, *AgApe* Central Apennines, *AgAlp* Eastern Alps, *Aclsr* Israel

Simmons (1980). Although our sampling included stocks that were assumed to be genetically pure (e.g., for Spanish red-legged partridges; J. Nadal, pers. comm., for Aulla breeding stock Baratti et al. 2005), some samples might have been affected by genetic introgression from other *Alectoris* species. In this case, they could well represent possible sources of introgressive hybridization for the Sardinian population.

DNA from muscle or feather was extracted using the QIAamp tissue kit (Qiagen GmbH, Hilden, Germany). Blood drawn from the wing vein was preserved in an anticoagulant solution of 0.25 M EDTA and used for DNA isolation employing the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN).

PCR-RFLP screening

Sardinian partridges were screened for maternal introgression by developing a PCR-RFLP method to discriminate among the four *Alectoris* species. Two pairs of PCR primers (AB1F: 5'-GACAACCCAACCCTTACCCGATT-3' and AB1R: 5'-TTTTCAAGGGTGCCAATTATG GG-3'; AB2F: 5'-CGCCTGATGAACTTCGGCTCC-3' and AB2R: 5'-TTGTTTGAGCCGGATTCGTGGA-3') were used to amplify two partially overlapping regions of the mitochondrial cytochrome *b* (*cytb*) gene that were, respec-

tively, 610 bp (fragment AB1) and 536 bp long (fragment AB2). Primers were designed to anneal to conserved sequences, taken from the alignment of published *cytb* sequences belonging to the four Mediterranean *Alectoris* species (GenBank accession nos.: Z48771 *A. barbara*, Z48772 *A. graeca*, Z48775 *A. rufa* (Randi 1996), and L08378 *A. chukar* (Kornegay et al. 1993)). On the basis of species-specific nucleotide differences and with the aim to obtain diagnostic restriction patterns, enzymes *HincII*, *Eco147I*, and *TasI* were selected to digest the AB1 fragment, while *AhaI* and *PstI* were employed to digest the AB2 fragment (Table 1). Multiple digestions were used to reduce the chance of misclassification due to homoplasy. Amplicon digestions were performed following the manufacturer's protocols (MBI Fermentas, Vilnius, Lithuania). After digestion, the restriction pattern obtained by electrophoresis in a 2.5% agarose gel (NuSieve 3:1, Cambrex, East Rutherford, NJ, USA) was compared to the patterns predicted according to the previously observed sequence differences (Table 1). In order to confirm each different restriction pattern (i.e., haplotype), the corresponding region was sequenced using both forward primers and the BigDye Terminator Cycle Sequencing 3.0 kit (Applied Biosystems, Foster City, CA). Sequencing runs were performed by capillary electrophoresis in an ABI PRISM 3130 automatic sequencer.

Table 1 Restriction scheme of the PCR-RFLP method developed to screen Sardinian partridges for the introgression of other species' mtDNA

Cytb region	AB1 (610bp)							AB2 (536bp)			
	Hinc II		Eco147I		Tas I			Alu I		Pst I	
	A		B		C/D			E		F	
Restriction pattern	1	0	1	0	1/0	0/0	0/1	1	0	1	0
Resulting fragment size (bp)	511	610	428	610	500	610	398	290	536	403	536
	99		182		110		212	246		133	
<i>A. barbara</i>	1		1		1/0			1		1	
<i>A. rufa</i>	0		0		0/0			0		0	
<i>A. chukar</i>	0		0		0/1			0		0	
<i>A. graeca</i>	0		0		0/0			0		0	

Restriction patterns obtained by digesting two fragments of the cytb gene (AB1 and AB2) with five different enzymes cutting at six potential sites (A to F) are shown. The restriction pattern is coded as 1 or 0 if the enzyme, respectively, cuts or does not cut the corresponding site, producing restriction fragments of the specified size

Microsatellite amplification

For cross-species amplifications, we used eight chicken microsatellites (Animal Breeding and Genetics Group 2008) that had been selected among those providing the best performance in different galliform species (Baratti et al. 2001, 2005) and appearing to be polymorphic in red-legged and chukar partridge populations (Baratti et al. 2005; Table 2). PCRs were carried out following the protocol described by Crooijmans et al. (1996). Fragments were amplified at different annealing temperatures (Table 2) and separated by 1.5% agarose gel electrophoresis. PCR products were combined on the basis of marker size and fluorescent dye, and loaded onto an ABI PRISM 310

automated sequencer; alleles were determined by GENESCAN and GENOTYPER softwares (Applied Biosystems).

Data analysis

In order to assess levels of genetic diversity in the Sardinian population, expected (H_E , unbiased gene diversity; Nei 1987) and observed heterozygosity (H_O) were calculated with GENETIX 4.05 (Belkhir et al. 2004). In addition, in order to account for differences in sample size, values of allelic richness (k_{AR}) were calculated for each population using HP-RARE (Kalinowski 2005) and setting rarefaction according to the smallest sample size in the computation.

Table 2 List of microsatellites used in the present study, with relative annealing temperature (T_a) and repeat motif (as detected in *Alectoris rufa*, Baratti et al. 2005)

Marker	T_a (°C)	Repeat motif	<i>A. barbara</i>		<i>A. graeca</i>		<i>A. rufa</i>		<i>A. chukar</i>	
			N_a	Size (bp)	N_a	Size (bp)	N_a	Size (bp)	N_a	Size (bp)
MCW043 ^a	58	(T) ₂₂	8	129–137	3	131–140	6	129–145	2	131–137
MCW044 ^b	58	(T) ₂₃	1	144	2	141–144	2	141–144	2	141–144
MCW104 ^a	55	(TG) ₁₄	4	81–105	9	93–111	4	81–105	6	81–107
MCW121 ^c	55	(TG) ₁₃	1	201	1	217	6	191–203	3	193–203
MCW146 ^c	60	(TG) ₁₂	8	154–170	5	132–154	3	154–158	2	154–158
MCW152 ^c	54	(AAAC) ₆	1	181	1	181	1	181	2	181–185
MCW199 ^c	54	(TG) ₅ (GC) ₂ (TG) ₁₁	8	278–292	8	280–298	5	282–292	3	278–282
MCW215 ^c	54	(TG) _n	2	248–250	5	258–278	8	254–276	5	250–264

Allele size range and number of detected alleles (N_a) are provided for each partridge species considered in the study. Letters refer to the source reporting marker specifications for *Gallus gallus*

^a Crooijmans et al. 1996

^b Crooijmans et al. 1995

^c Crooijmans et al. 1997

Deviations from the Hardy–Weinberg equilibrium (HWE) were tested in the population by GENEPOP 4 (Raymond and Rousset 1995) that employs a probability exact test based on a Markov chain method (Guo and Thompson 1992). Whenever multiple tests were performed, nominal levels of significance were lowered using the Bonferroni correction (Sokal and Rohlf 1995). Observed deviations from HWE were evaluated for the possible effect of inbreeding by calculating F_{IS} and testing its significance over 1,000 permutations of alleles in GENETIX.

Signs of recent population bottlenecks were investigated using allele frequency data and the Wilcoxon sign-rank test (Luikart and Cornuet 1998) in BOTTLENECK 1.2 (Cornuet and Luikart 1996). Three mutation models were tentatively used: the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phased model (TPM), defined with 70% of SMM and 30% of IAM.

The extent of genetic differentiation between the Sardinian and Tunisian populations at the eight nuclear loci was assessed by calculating the overall F_{ST} (Weir and Cockerham 1984) with its 95% confidence intervals ($CI_{95\%}$) and comparing it to the values obtained from comparisons among all populations. In addition, in GENETIX, genotypes were clustered by factorial correspondence analysis (FCA; Benzécri 1973) and plotted on a three-dimensional space, where axes were represented by the three main factors (i.e., those with the highest cumulative inertia).

Finally, a Bayesian clustering procedure implemented in STRUCTURE 2.1 (Pritchard et al. 2000) was applied with the aim to (1) verify whether any individual in the Sardinian sample could be an immigrant into the island or had recent immigrant ancestors, (2) classify individuals from Sardinia into one or more parental species by checking for possible signs of genetic admixture (i.e., hybridization). The software was used with a two-step approach as described by Pritchard and Wen (2004).

First, a direct assignment test was performed assuming no admixture and, thus, supposing a partition of the four different species into four discrete clusters ($K=4$). Here, we considered the possibility that specimens collected in Sardinia clustered with species other than *A. barbara*.

Second, a “blind” cluster analysis was performed using the admixture model (i.e., assuming that individuals may have admixed ancestry) and neglecting any prior information on the population from which individuals had been sampled. In this case, we focused on the consistency between the Bayesian grouping pattern and the predefined individual classification (i.e., the division into four different species). This explorative analysis was performed with K raising from 1 to 10, testing the hypothesis that the optimal K , i.e., the smallest value of K that captures the major structure in the data, was equal to four (number of species).

Five runs were performed for each given value of K to check for consistency across runs.

In both cases, simulations in STRUCTURE were performed with 10^5 burn-in iterations and 10^5 iterations for data collection, using independent allele frequencies among populations ($\lambda=1$). The selected burn-in length warranted the convergence of likelihood estimates.

Results

Genetic variation, Hardy–Weinberg equilibrium, and genetic differentiation

The selected microsatellites appeared to be conserved within the genus, since cross-species amplifications were achieved for all loci screened. All markers that had proven to be polymorphic in *A. rufa* and *A. chukar* (Baratti et al. 2005) were also amplified in *A. graeca* and *A. barbara*. In the putatively assigned barbary partridges (cumulative sample from Tunisia and Sardinia), five out of eight loci were polymorphic, while the remaining three (*MCW044*, *MCW121*, and *MCW152*) had fixed alleles. In rock partridges, two loci proved to be monomorphic (*MCW121* and *MCW146*).

The overall number of detected alleles across populations was 71. Their number varied greatly among loci, ranging from two (*MCW044*) to 12 (*MCW215*). Medium to low levels of heterozygosity were obtained at the typed loci for all the sampled populations ($H_E=0.293$ – 0.600 , $H_O=0.160$ – 0.424), with the highest values being observed in Spanish *A. rufa* and the lowest ones seen in Alpine *A. graeca* (Table 3). When compared to the other samples, the Sardinian population had the lowest number of polymorphic loci (only four) and a low genetic diversity ($H_E=0.310$, $H_O=0.273$), even with respect to allelic richness (k_{AR} AbSar=2.64, range for all other samples: $k_{AR}=2.47$ – 3.90). The low variability do not seem to be affected by the inclusion of captive-reared individuals, as the specimens from breeding stations in the island had a higher average heterozygosity than free-living individuals ($H_E=0.305$ vs. 0.421 , respectively).

The Sardinian sample suffered from a lack of heterozygotes with respect to HWE expectations, producing a significant deviation from the equilibrium ($p<0.001$). Indeed, the overall F_{IS} value (0.120) was significant ($p<0.01$). An excess of gene diversity, with respect to that predicted according to the actual number of alleles, was revealed by the analysis in BOTTLENECK, both under the IAM and the TPM mutation models (Wilcoxon sign-rank test, $p=0.031$ for both models). Such results suggest that the Sardinian population may have undergone a recent bottleneck.

Table 3 Estimates of genetic variation calculated for the Sardinian partridge population and other Mediterranean samples

Population	Sample size	H_E	H_O	k_{AR}	F_{IS}
AbSar	65	0.310	0.273	2.64	0.120 **
AbTun	10	0.395	0.350	2.91	0.120 <i>ns</i>
ArAul	17	0.455	0.390	2.75	0.147 *
ArSpa	10	0.600	0.424	3.90	0.305 **
AgSic	10	0.320	0.188	2.47	0.428 **
AgApe	20	0.461	0.317	3.53	0.319 **
AgAlp	10	0.293	0.160	2.90	0.467 **
AcIsr	17	0.390	0.265	2.91	0.326 **

Significance of F_{IS} values was tested over permutations in GENETIX
 H_E expected heterozygosity; H_O observed heterozygosity; k_{AR} allelic richness, *ns* not significant

* $p < 5\%$, ** $p < 1\%$

When compared to the Tunisian sample, Sardinian partridges showed a fixed allele at locus *MCW104*, while they shared the same alleles fixed at loci *MCW044*, *MCW121*, and *MCW152* in the African population. Diversity values of AbSar were all lower than those obtained for AbTun ($H_E=0.395$, $H_O=0.350$, $k_{AR}=3.00$), even though no statistical significance was found (paired *t* test, $df=7$, for all comparisons $0.2 > p > 0.05$). The value of F_{ST} between Sardinian and Tunisian partridges was 0.204 ($CI_{95\%}$ 0.183–0.589), less than half of those estimated between the Sardinian sample and the other populations taken into account, ranging from 0.495 ($CI_{95\%}$ 0.355–0.699, AbSar vs. ArAul) to 0.615 ($CI_{95\%}$ 0.433–0.796, AbSar vs. Ach).

Genetic composition of Sardinian partridges

The combination of the five enzymes in Table 1 provided a single RFLP haplotype in all the Sardinian specimens corresponding to that predicted for barbara partridges on the basis of the available GenBank *cytb* sequence. Thus, they were unambiguously attributed to the *A. barbara* lineage. Therefore, there was no evidence of exotic mtDNA lineages in the matrilineal component of the population. However, such result did not exclude introgression into the nuclear genome. The analysis of microsatellite data allowed to clarify this point too.

Individual multilocus genotypes plotted against the three major components that were identified by the FCA (cumulative inertia=23%) showed a sharp separation into four clusters, roughly corresponding to the four species (Fig. 2). Only red-legged and chukar partridges did partially overlap. The first component (CA-I) alone, explaining 9.3% of the variance, sharply separated *A. barbara* from *A. graeca* genotypes; on the other hand, CA-II fully resolved *A. barbara* with respect to *A. rufa* and *A. chukar* samples. Sardinian and Tunisian partridges were included in the same cluster, forming two adjacent subgroups.

Analogously to FCA, the direct assignment test, performed with STRUCTURE under the hypothesis of no genetic admixture, classified all partridges collected in Sardinia into the cluster corresponding to *Alectoris barbara* (Fig. 3A).

The “blind” Bayesian analysis was performed for $K=1-10$, implementing the admixture model (i.e., assuming the possibility of crossbreeding between different taxa). Results of simulations in terms of LnP(D) are shown in

Fig. 2 Three-dimensional plot resulting from the factorial correspondence analysis (FCA, Benzécri 1973) of partridge multilocus genotypes. The three principal factors, explaining 23.1% of the overall variation, were used to draw the plot. Ellipses delimit the four putative species included in the sampling: *Alectoris rufa*, *A. chukar*, *A. graeca*, and *A. barbara* (Tunisian sample in blue and Sardinian sample in yellow)

Fig. 3 Proportions of membership of each genotyped individual to the four clusters inferred by Structure. **A** Assignment test performed considering population data and excluding the possibility of genetic admixture. **B** Blind simulation performed neglecting prior population

information and admitting genetic admixture among the populations. In both cases, results refer to simulations performed over 100,000 iterations with $K=4$, assuming independent allele frequencies among populations

Fig. 4. The most likely subdivision resulted to be $K=4$, as the likelihood difference between $K=4$ and $K=3$ was far higher than that between $K=5$ and $K=4$. Each of the four samples corresponding to the four putative species had an average proportion of membership to one of the four inferred clusters $Q>0.95$. Tunisian barbary partridges were assigned to cluster I ($Q_I=0.96$). Red-legged, chukar,

and rock partridges corresponded to clusters II, III, and IV, respectively ($Q_{II}=0.97$, $Q_{III}=0.96$, $Q_{IV}=0.98$). The mean value of Q_I for Sardinian partridges was equal to 0.98. Only one specimen out of 65 had $Q_I<0.90$ (Fig. 3B); this was a wild partridge from Northern Sardinia, which possibly had hybrids with red-legged partridges in its ancestry ($Q_{II}=0.094$).

Fig. 4 Posterior probability of the data ($\ln [P(D|K)]$) as a function of K (number of clusters), as resulting from the simulations in Structure with *Alectoris* microsatellite data. Mean and standard deviation (vertical bars) were computed over five independent simulations, performed under the admixture model (100,000 iterations of both burn-in and data collection)

Discussion

The present study provides insights into the genetic status of the Sardinian partridge population. Low levels of overall genetic variation are detected in Sardinia. In our study, four loci (50%) proved to be monomorphic in the Sardinian population, and a low value of allelic richness was obtained for the remaining four. This result confirms the one reported by Randi et al. (1992) who detected less electrophoretic polymorphisms in Sardinian *A. barbara* when compared to seven other Mediterranean partridge populations. Only 6.1% of the allozyme loci ($n=33$) assayed by them were polymorphic in Sardinian partridges, whereas this proportion ranged between 12.1% and 39.4% in the other investigated populations. Partridge populations inhabiting other Mediterranean islands have been shown to have low genetic diversity at microsatellite loci (e.g., Sicily, Randi et al. 2003, and Cyprus, Tejedor et al. 2005). Both demographic

events (i.e., bottlenecks) and extensive release of captive-reared birds to reinforce low-density populations could account for the observed levels of variability.

There was limited divergence between the Sardinian population and North African conspecifics. The estimated F_{ST} value, the relatively low distance in the FCA plot, and the fact that the two populations shared the same fixed alleles at three loci are all consistent with the hypothesis of a historical human-mediated introduction. If true, the present level of differentiation could be a consequence of the post-foundation genetic drift of the insular stock or might suggest that the founders came from an area in North Africa different from Tunisia.

No relevant sign of a recent introduction of exotic partridges belonging to *Alectoris* species other than *A. barbara* was detected either in the wild population or in the sampled captive stocks. Our analysis relied on the use of eight microsatellites which warranted a good resolution power and splitted the specimens belonging to the four *Alectoris* species into four discrete clusters (see Figs. 2, 3). Although the number of markers is quite smaller than that suggested by Vähä and Primmer (2006), a set of few markers is sufficient to reveal hybrids (F1 and F2) if the parental populations diverge notably (Boecklen and Howard 1997; Barilani et al. 2007b). This is the case here, as the detected divergence between parental *A. barbara* (AbTun) and the other putative parental species was quite high (F_{ST} values ranged between 0.350 and 0.515). The Bayesian assignment tests conducted without any prior information on the population detected only one Sardinian specimen out of 65 with $Q_1 < 0.90$ (i.e., a probability of less than 90% to belong to the cluster identified as *A. barbara*). This proved to be a wild partridge from Northern Sardinia, showing a limited (10%) trace of *A. rufa* DNA in its ancestry. This result strikingly differs from those recently reported for other European partridge populations, where human-caused wide-scale hybridization seemed to be the rule (Negro et al. 2001; Baratti et al. 2005; Barbanera et al. 2005; Barilani et al. 2007a, b; Tejedor et al. 2007). In addition, the analysis of individuals sampled in four breeding stations showed levels of genetic variation similar to that in the wild population, while no sign of hybridization was found.

Following such encouraging outcomes, we believe it necessary that a wider and constant monitoring of both the wild Sardinian population and the breeding stocks be carried out in order to exclude the occurrence of hybrids in yet nonsampled areas of Sardinia and to prevent genetic pollution in the future.

Implications for management and conservation

The barbary partridge is listed as Species of European Conservation Concern (SPEC) 3 in the SPEC list, but the

actual status of *A. barbara* populations and their main threats are poorly known (Tucker and Heath 1994). The future status of the Sardinian population will be influenced by two main factors: the depletion of its genetic diversity and the risk of genetic pollution by introgressive hybridization with exotic species. While signs of genetic impoverishment were found in the wild population, possibly due to local inbreeding or to extensive restocking with inbred captive-reared birds, no remarkable traces of exotic genes were detected in the present study.

Unlike the Native Sicilian partridge population, which meets the requirements for an evolutionary significant unit (Randi et al. 2003), the Sardinian population could only represent a management unit (Moritz 1994), in that it appears to be the result of an anthropogenic introduction and that it shares a recent common ancestry with North African conspecific populations. Nonetheless, by virtue of the social interest towards this species in Sardinia, its long-term conservation represents a priority for the management of the Sardinian fauna.

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