

Phylogeography and founder effect of the endangered Corsican red deer (*Cervus elaphus corsicanus*)

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Abstract Red deer ($n = 149$) from eight geographical locations, including the endangered endemic populations from the Tyrrhenian islands (Sardinia and Corsica), were analysed at eight polymorphic microsatellite loci. Two questions were addressed: (1) Is there a founder effect in the Corsican population, which was reintroduced to the island using Sardinian deer after the species' extinction on Corsica? (2) What is the origin of the Tyrrhenian or Corsican red deer (*Cervus elaphus corsicanus*)? Our results showed signs of a founder effect for the red deer on Corsica in that these deer showed differentiation from the Sardinian population as measured by F_{ST} values, assignment tests (with and without a priori definition of populations) and individual-based dendrograms. Genetic variability, however, did not differ significantly between the two populations. With respect to the phylogeography of *C. e. corsicanus* we found that both deer from North-Africa and Mesola on the Italian mainland were genetically close to the Corsican red deer, but phylogenetic trees based on genetic distances were only poorly supported statistically. Among all populations studied the Mesola red deer showed the lowest distance values from Corsican red deer and yielded allele frequencies that were more similar to those of *C. e. corsicanus* than were those of North-African red deer. These results are in line with recent palaeontological and archaeozoological findings which suggest that the Corsican red deer is derived from small Italian red deer introduced from the mainland to Sardinia and Corsica during the Late Neolithic and just before the beginning of Classical

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Antiquity, respectively. They also suggest a possible recent introduction of Tyrrhenian red deer to North-Africa (rather than the other way around), thus accounting for the close genetic relationship (especially based on mitochondrial DNA) that has repeatedly been found between *C. e. corsicanus* and *C. e. barbarus*.

Keywords *Cervus elaphus corsicanus* · Conservation genetics · Corsica · Microsatellites · Sardinia

Introduction

The Corsican red deer (*Cervus elaphus corsicanus* Erxleben, 1777) is not only the smallest of red deer but also one of the most threatened cervid taxa of the world. It is confined to the Tyrrhenian islands of Sardinia and Corsica and listed as endangered by the IUCN (IUCN Red List 2007, Wemmer 1998). Recent population and conservation genetic studies revealed very low levels of genetic variability in the Corsican red deer, in particular at nuclear microsatellite loci (Zachos et al. 2003; Lorenzini et al. 2005; Hmwe et al. 2006a), but over the last decades the deer have recovered from a bottleneck of only 100–150 remaining animals, numbering at least 2,000–2,300 head at the end of the twentieth century (Krumbiegel 1982; Mattioli et al. 2001; Lovari et al. 2007; Banwell (1998) gives an estimate of 1,500 head). After the red deer had become extinct on Corsica around 1970 (Vigne and Marival-Vigne 1988), the Corsican population was refounded with altogether 13 individuals (four males and nine females) introduced from Sardinia in 1985, 1987 and 1994 (Dolan 1988; Gonzalez and Kidjo 2002; Kidjo et al. 2006; Gonzalez personal communication). A fourteenth specimen, a male, died a few days after its introduction (Kidjo personal communication). These animals were kept in breeding enclosures, and since 1998 there have been repeated releases of deer into the wild resulting in a free-ranging population of about 200 head in 2005 (Kidjo et al. 2006).

The origin of *C. e. corsicanus* has been a matter of considerable debate (reviewed in Vigne 1988; Zachos and Hartl 2006). There are basically two unresolved problems—where exactly did it come from, and how did it reach the Tyrrhenian islands, naturally or through human introduction? The human introduction hypothesis was already put forward by Lydekker (1898), and Groves (personal communication to Geist 1998) thought that the Corsican red deer was derived from the North-African Barbary red deer (*C. e. barbarus*) and introduced to the Tyrrhenian islands by the Phoenicians. In accordance with this, Ludt et al. (2004), in a comprehensive phylogeographic study based on cytochrome *b* sequences, found close relationships between Sardinian and Barbary red deer, which together formed a clade quite distinct from the rest of the Eurasian red deer studied. Introduction from North-Africa to Sardinia has very probably occurred in other mammal species such as hares (*Lepus* sp., Scandura et al. 2007). It has also been hypothesized that Corsican, Barbary and Spanish (*C. e. hispanicus*) red deer are closely related (Flerov 1952), which was partly supported for Spanish and Corsican deer by Zachos et al. (2003) and Hmwe et al. (2006a) based on sequences of the mitochondrial control region, but other studies or markers did not confirm these results (Zachos et al. 2003; Ludt et al. 2004; Hmwe et al. 2006a). Yet another genetically based hypothesis was put forward by Hartl et al. (1995) who favoured the Near East as the place of origin of *C. e. corsicanus* because the Sardinian deer they studied showed mitochondrial RFLP affinities to Bulgarian red deer. This latter population might be phylogenetically similar or even identical to the eastern subspecies *C. e. maral* (see Groves and Grubb 1987), but none of the subsequent genetic studies corroborated this hypothesis.

Interestingly, preliminary results based on nuclear microsatellite markers showed close relationships between *C. e. corsicanus* and European mainland red deer (Zachos et al. 2003), in particular those from Mesola in the Po delta (Hmwe et al. 2006a). The Mesola population is special in that it is probably the only truly autochthonous red deer population in the Italian peninsula and thus, it may represent a remnant of the original Italian gene pool (Mattioli 1990; Mattioli et al. 2003). This is in line with close morphological similarities between Holocene bone series from southern Italy and nineteenth and early twentieth century samples from the now extinct original Corsican red deer population (Vigne 1988) and would suggest an introduction of Italian red deer to Sardinia and Corsica or even a natural colonization of the Tyrrhenian islands from mainland Italy, possibly during the maximum of the last (or an earlier) glacial when the sea-level was up to 130 m lower than today (Shackleton 1987). The hypothesis of a natural colonization, however, stands in clear contrast to palaeontological and archaeozoological data (Section “Discussion”). Unfortunately, Hmwe et al. (2006a) did not include Barbary deer into their analysis to directly compare the nuclear genetic affinities of the Corsican red deer to Mesola deer on the one hand and African deer on the other.

Most phylogeographic studies are carried out using mitochondrial sequences such as the control region or the cytochrome *b* gene. There is, however, a growing body of evidence that these markers are in many cases too conservative to unravel recent demographic events at or below the species level (cf. Cronin 1993; Taberlet et al. 1998; Funk and Omland 2003; Hmwe et al. 2006a). This means that coalescent times of the sequences studied may considerably predate the time of population sundering resulting in a systematic mismatch of gene trees and population trees (Avice 1989, 2000; Pamilo and Nei 1988). Microsatellites, with their very high mutation rates, may be well suited to close this gap between genetic and population “coalescence” and have successfully been used to solve phylogeographic questions in cervids (Goodman et al. 2001). These markers are short tandem-repetitive DNA units which have often been used in population genetic analyses before (e. g., Estonba et al. 2006; Hmwe et al. 2006b; Kalz et al. 2006; Nussey et al. 2006; Redeker et al. 2006; Zachos et al. 2006). In the present study, we analysed for the first time Corsican red deer together with their potentially phylogenetically closest populations (Mesola, representing Italy, northern Africa and Spain) by using a panel of polymorphic microsatellite loci. To make the sampling more comprehensive we also included red deer from several further, taxonomically distinct European populations. In addition, since we had samples from both Sardinia and Corsica, we used our data to address the question of a possible founder effect in the reintroduced Corsican population as compared to its population of origin on Sardinia.

Methods

Sampling

A total of 149 red deer samples was collected from eight different sites in Europe and North-Africa (Fig. 1). We collected tissue samples from culled animals where the species is hunted (Scotland, France, Spain and Romania) or obtained blood samples taken by jugular puncture from live-caught animals (Sardinia and Mesola, cf. Hmwe et al. 2006a). The Corsican samples consisted of pieces of ear tissue sampled when the animals were individually marked for a monitoring project. For details on the sampling of Tunisian Barbary deer see Hajji et al. (2007).



Fig. 1 Geographical location of the red deer populations studied. 1 = Dunachton (Scotland, $n = 9$), 2 = Chambord (France, $n = 14$), 3 = southwestern Spain ($n = 21$), 4 = Tunisia ($n = 30$), 5 = Sardinia ($n = 23$), 6 = Corsica ($n = 10$), 7 = Mesola (Italy, $n = 25$), 8 = Carpathians (Romania, $n = 17$)

The taxonomic designation of the populations is given in Table 2. There have been many attempts at classifying red deer into different subspecies, and although the subspecies concept is partly arbitrary and hence not useful as an evolutionary unit (Mayr 1963), subspecific names may serve as terms to designate geographically, morphologically or genetically different populations. In the case of the red deer, the subspecies listed in Table 2 are all morphologically differentiated although this does not necessarily imply that the deer from France and Mesola (both classified as *C. e. hippelaphus*) are genetically and historically closer to one another than members of different subspecies. It should be made clear that the present study cannot serve as a general systematic analysis of red deer subspecies. For such an analysis to be valid the subspecific designations would first have to be genetically substantiated, and to achieve this, a much more extensive sampling would be necessary in order to cover the whole range of “intra-subspecific” variability. The present choice of populations merely aimed at including geographically and genetically diverse specimens. The taxonomic designations follow Dolan (1988) except for the Carpathian red deer which we separated from the Central European red deer since its genetic distinctness has recently been confirmed (Feulner et al. 2004). Apart from Scandinavian populations or subspecies our sampling thus comprises the great majority of European-type red deer.

Molecular analysis

DNA extraction from tissue samples was carried out either using the Dneasy Tissue Kit (QIAGEN, Hilden, Germany) or following the standard method of proteinase-K digestion, phenol chloroform extraction and precipitation in ethanol (Sambrook and Russel 2001). DNA from faecal material (some of the Tunisian samples) was extracted by means of the QIAGEN QIAMP^R DNA stool mini Kit.

Individual genotypes were established through amplification of eight polymorphic microsatellite loci: INRA 121, OarVH110, BL42, BM203, BM757, BMC1009, IDVGA55

and TGLA53 (for further information on these loci and amplification conditions see Bonnet et al. 2002). Due to the comparatively low amount of DNA extracted from the non-invasive Tunisian samples and in order to reduce the risk of overlooking null alleles and large allele dropout we carried out the PCR and allele determination for each Tunisian specimen twice for skin samples, four times for hair samples and six times for faecal samples (multiple-tubes approach, see Taberlet et al. 1996; see also Hajji et al. 2007). Length determination of the microsatellite alleles was conducted with automatic sequencers and the Genescan and Genetic profiler software.

Statistical analysis

The eight loci were tested for pairwise linkage disequilibrium (for each population and over all populations) using the GENEPOP software (Raymond and Rousset 1995). In addition to the multiple-tubes approach we tested our data for null alleles, large allele dropout and stutter bands with the MICRO-CHECKER programme (van Oosterhout et al. 2004) because these potential error sources are known to distort allele frequencies and estimates of heterozygosity (Pemberton et al. 1995; Wattier et al. 1998; Ewen et al. 2000). Especially with null alleles, however, these tests can only serve as a vague indication since null allele probabilities are based on heterozygote deficiencies which are also caused by a variety of other factors such as inbreeding or population substructuring.

To give a general population genetic characterization of the populations studied we carried out the following calculations. Observed (H_O) and expected (H_E) heterozygosity as well as deviations from Hardy-Weinberg-Equilibrium (HWE) were computed with Arlequin (Schneider et al. 2000). Values of allelic richness were computed with the FSTAT software (Goudet 1995) as a measure of allelic diversity corrected for differences in sample size. Private alleles (alleles present exclusively in one population) were counted and their relative proportions calculated. To gain insight into the total genetic variability of *C. e. corsicanus* we also calculated variability parameters for the pooled data set of Corsica and Sardinia. To examine the possible differentiation between the Corsican red deer and its population of origin from Sardinia we looked for alleles present in one but not in the other population and tested for significant differences in variability parameters (observed and expected heterozygosity, allelic richness) between Corsica and Sardinia by means of Wilcoxon matched-pairs signed-ranks tests. Apart from this and the F_{ST} -based test of population differentiation (see below) we also constructed an individual-based dendrogram with the neighbor-joining algorithm and 1000 bootstrap replicates implemented in the PHYLIP software (Felsenstein 1993) to see if the Corsican deer cluster together relative to the Sardinian ones. Pairwise genetic distances between individuals from Sardinia and Corsica were quantified by (1-Dps) distances (see below). Finally, we used the Bayesian approach of testing for population subdivision without a priori definition of populations implemented in STRUCTURE (Pritchard et al. 2000). The analysis was run under admixture conditions with a burn-in length of 100,000 and a simulation length of 1,000,000 repetitions testing for a subdivision into two different groups.

Differentiation among all populations was tested for with pairwise F_{ST} -values. Overall F_{ST} - and R_{ST} -values (the latter being a microsatellite-specific analogue of F_{ST} based on the stepwise mutation model) were calculated with Arlequin as a measure of the amount of genetic variation accounted for by differentiation among (as opposed to variability within) populations.

The question of the phylogeographic origin of the Corsican red deer was statistically addressed as follows. The proportions of *C. e. corsicanus* alleles present in the other

populations were calculated. Assignment tests based on individual genotypes were conducted on the 149 individuals using the Bayesian and the frequency approach as implemented in GENECLASS (Piry et al. 2004). All individuals are assigned to the population of which their genotype is most typical so that incorrect assignments indicate genetic similarity of the respective populations. As in a previous analysis on the phylogeography of the Corsican red deer (Hmwe et al. 2006a) genetic differentiation was further quantified with pairwise Nei's (1972) standard distances D , Cavalli-Sforza and Edwards' (1967) chord distances (both computed with PHYLIP) and with the proportion-of-shared-alleles-distances D_{ps} (Bowcock et al. 1994), calculated as $(1-D_{ps})$ with the programme MICRO-SAT (Minch et al. 1995). D_{ps} is based on the sums of allele frequencies shared between populations and has been shown to be especially suitable for resolving relationships of closely related taxa with microsatellite data (Cornuet et al. 1999). The distance values were used for tree reconstruction with the neighbour-joining algorithm of the PHYLIP package. Support for nodes was assessed by 1000 bootstrap replicates. The calculation of genetic distances and tree reconstruction was conducted twice—with Corsica and Sardinia as separate populations and with the two samples pooled as *C. e. corsicanus*.

The significance level of 0.05 was adjusted using Bonferroni corrections (Rice 1989) whenever multiple tests were carried out.

Results

There was no linkage disequilibrium between any two loci, neither in each single population nor over all populations pooled. Therefore, all loci were included in the multi-locus calculations. There was no evidence of large allele dropout in our data set. In one population (Carpathians) locus OarVH110 showed signs of allele frequency distortion through PCR stuttering. Evidence of null alleles was found for single loci in all eight populations, especially in Tunisia and the Tyrrhenian islands (Corsica and Sardinia), which are known to be inbred. Significant deviations from HWE due to an excess of homozygotes occurred in most populations for single loci (the maximum was Tunisia with five loci not at HWE), but none of the loci showed deviations in all populations. In many, but not all cases the loci with significant deviations matched those for which the MICRO-CHECKER analysis suggested the possibility of null alleles.

Values of observed and expected heterozygosity as well as allelic richness and data on private alleles are given in Table 1. We found eight alleles in Corsica that were absent from our Sardinian sample while 21 Sardinian alleles were not found in Corsica. The Wilcoxon tests for differences in variability parameters between Corsica and Sardinia did not yield significant results (observed heterozygosity: $P = 0.889$, expected heterozygosity: $P = 0.293$, allelic richness: $P = 0.206$). However, while for the single populations deviations from HWE were only found at two loci for Sardinia (and at none for Corsica), the pooled data set yielded significant deviations due to an excess of homozygotes at five loci.

Both overall F_{ST} (0.202) and R_{ST} (0.317) were significantly different from zero, indicating that about 20% or 32% of the genetic variance were accounted for by differentiation among populations and 80% or 68% by variability within them. Pairwise F_{ST} -values were all significantly different from zero showing that all population were genetically different from one another. The value between Sardinia and Corsica was 0.151. The assignment tests confirmed these results in that both methods correctly assigned 145 out of 149 individuals to their population of origin. The frequency-based approach incorrectly assigned two Sardinian deer to Corsica, one Scottish deer to the Carpathians and one Carpathian specimen to

Table 1 Genetic variability and private alleles of the red deer populations studied

Population	N	H _O	H _E	AR	A	PA
<i>C. e. cors.</i>	33	0.53	0.72	4.6	51	8 (15.7%)
Corsica	10	0.54	0.64	3.5	30	1 (3.3%)
Sardinia	23	0.53	0.71	4.4	44	7 (15.9%)
Tunisia	30	0.47	0.75	4.3	39	1 (2.6%)
Spain	21	0.63	0.81	6.1	64	11 (17.2%)
France	14	0.63	0.77	5.1	46	1 (2.2%)
Mesola	25	0.47	0.60	3.1	32	5 (15.6%)
Scotland	9	0.69	0.79	6.2	51	7 (13.7%)
Carpathians	17	0.61	0.84	7.1	73	10 (13.7%)

H_O and H_E: observed and expected heterozygosity, AR: allelic richness. N = sample size. H_O, H_E and AR are calculated over all eight loci. A = total number of alleles, PA = number of private alleles (% of total allele number given in parentheses). *C. e. cors.* = Corsica and Sardinia pooled

Scotland. The Bayesian test incorrectly assigned the same two Sardinian samples to Corsica and the same Carpathian deer to Scotland as the frequency-based test but additionally assigned one Corsican deer to Sardinia. The STRUCTURE analysis also yielded results indicating differentiation between Corsican and Sardinian red deer: all 10 Corsican specimens were assigned to group one (probability ranging from 79% to 98%), while only six out of 23 Sardinian individuals were assigned to this group (with lower probabilities ranging from 53% to 90%); the other 17 Sardinian deer were assigned to group two with probabilities between 75% and 98%. Although the two groups are not reciprocally exclusive with respect to the assignment of Corsican and Sardinian individuals, the results clearly show a non-random assignment of the red deer from the two islands indicative of differentiation between Corsica and Sardinia.

The number of *C. e. corsicanus* alleles present in the other populations along with their relative proportions (calculated as the percentage of all *C. e. corsicanus* alleles and as the percentage of alleles present in each population) are shown in Table 2. A graphic representation of allele frequencies at five loci for *C. e. corsicanus* (Sardinia and Corsica pooled) and the two sites probably closest to it, Tunisia and Mesola, is given in Fig. 2. From the histograms it is obvious that, in terms of allele distribution and frequencies, Mesola is more similar to the Corsican red deer than Tunisia.

Pairwise genetic chord and (1–Dps) distances are shown in Table 3. Nei's standard distances are not shown because their relative values were very similar to those of the other

Table 2 Allelic similarity between *C. e. corsicanus* and the other populations studied

Population	Subspecies	A	A _{cors}	P _{cors} [%]	R _{cors} [%]	Nei	Chord	1-Dps
Tunisia	<i>C. e. barbarus</i>	39	23	45.1	59.0	1.015	0.126	0.640
Spain	<i>C. e. hispanicus</i>	64	19	37.3	29.7	1.132	0.157	0.739
France	<i>C. e. hippelaphus</i>	46	23	45.1	50.0	0.730	0.110	0.588
Mesola	<i>C. e. hippelaphus</i>	32	17	33.3	53.1	0.745	0.117	0.585
Scotland	<i>C. e. scoticus</i>	51	18	35.3	35.3	1.350	0.158	0.777
Carpathians	<i>C. e. montanus</i>	73	26	51.0	35.6	1.330	0.145	0.748

Subspecies = subspecific designation according to present taxonomy, A = total number of alleles found, A_{cors} = number of alleles also found in the Corsican red deer, P_{cors} = proportion of all *corsicanus* alleles (i.e. A_{cors} divided by 51), R_{cors} = relative occurrence of *corsicanus* alleles in each of the other populations (i.e. A_{cors} divided by A). Nei, Chord and 1-Dps refer to Nei's (1972) standard, chord and 1-Dps distances between the listed populations and *C. e. corsicanus* (Corsican and Sardinian samples pooled)

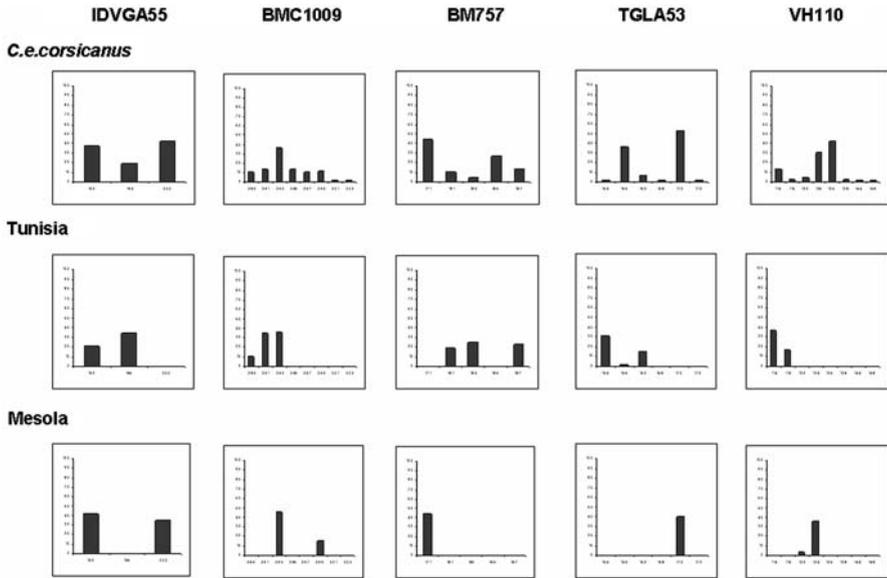


Fig. 2 Comparative allele frequencies at five microsatellite loci for the Corsican red deer, Tunisia and Mesola. Horizontal axis: allele length, vertical axis: frequency. The histograms are arranged in such a way that the same alleles are located below each other for direct comparison. The five columns of histograms refer to the loci, the three lines to populations

Table 3 (1–Dps) (above diagonal) and Cavalli-Sforza and Edwards’ chord (below diagonal) distances between red deer populations based upon allele frequencies of eight polymorphic microsatellite loci

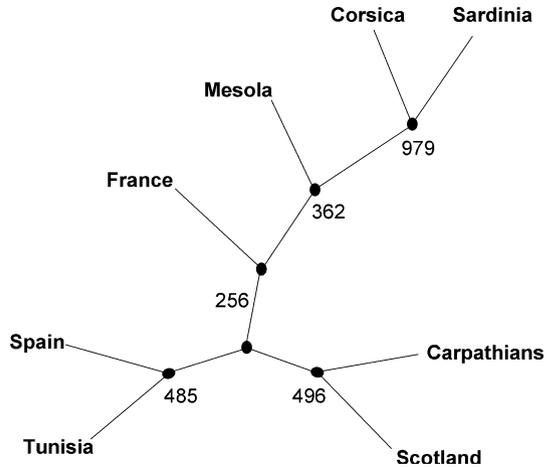
	COR	SAR	TUN	SPA	FRA	MES	SCO	CAR
COR	–	0.503	0.647	0.800	0.671	0.650	0.851	0.813
SAR	0.067	–	0.685	0.747	0.618	0.588	0.767	0.764
TUN	0.126	0.141	–	0.680	0.785	0.752	0.778	0.762
SPA	0.165	0.163	0.135	–	0.647	0.785	0.726	0.666
FRA	0.128	0.120	0.163	0.135	–	0.763	0.688	0.737
MES	0.129	0.120	0.160	0.165	0.155	–	0.795	0.634
SCO	0.175	0.162	0.170	0.150	0.142	0.169	–	0.643
CAR	0.159	0.151	0.153	0.135	0.139	0.122	0.120	–

COR = Corsica, SAR = Sardinia, TUN = Tunisia, SPA = Spain, FRA = France, MES = Mesola, SCO = Scotland, CAR = Carpathians

two distance measures (smallest value between Corsica and Sardinia; Tunisia, France and Mesola closest to Corsica and Sardinia; small values between Tunisia and Spain etc.). Distance values between the pooled *C. e. corsicanus* samples and the other populations are given in Table 2.

The trees yielded similar topologies for all three distance measures and irrespective of whether Sardinia and Corsica were combined or not (Fig. 3). The only well-supported node, however, was the one linking Sardinia and Corsica (72–98% bootstrap support). Tunisia and Spain on the one hand and Scotland and the Carpathians on the other were paired in all trees with low support (35–61%). In line with the distance values given in

Fig. 3 Neighbor-joining tree based on pairwise chord distances derived from allele frequencies at eight microsatellite loci. Numbers at nodes denote support from 1000 bootstrap replicates



Tables 2 and 3 the population closest to *C. e. corsicanus* was either Mesola (Dps trees and chord distance tree with Sardinia and Corsica treated separately) or France (standard distance trees and chord distance tree with Sardinia and Corsica combined), but all of these clades yielded bootstrap supports of less than 50%.

The individual-based Dps-tree for Sardinia and Corsica (not shown) yielded two main branches, one consisting of 18 Sardinian deer and one combining all 10 Corsican individuals with five Sardinian deer. The latter contained a subcluster of seven Corsican and two Sardinian individuals. Bootstrap support, however, was low for all nodes.

Discussion

The present study encompasses, for the first time, red deer from North-Africa, Mesola, Corsica and Sardinia in one microsatellite analysis aiming at elucidating the phylogeographic origin of *C. e. corsicanus*, one of the world's most threatened deer taxa (Dolan 1988). At the same time, it is also the first genetic analysis of the recently re-founded Corsican red deer population, making it possible to study the founder effect in this population by comparing its genetic characteristics to those of its population of origin on Sardinia.

Although one cannot totally rule out the possibility of the presence of null alleles in our data set, the multiple-tubes approach in our Tunisian samples and the fact that the calculated probability of null alleles was particularly high in populations known to be bottlenecked and inbred favour the conclusion that the detected heterozygote deficiencies and deviations from HWE are better explained by inbreeding than by null alleles.

Founder effect in the Corsican population

C. e. corsicanus clearly still shows the signature of its past bottleneck—as do the populations from Tunisia and Mesola. These three stocks exhibit low or very low genetic variability in a European comparison (see, apart from the other populations in this study, e.g., Zachos et al. 2003, 2007; Feulner et al. 2004; Hmwe et al. 2006a), and antler malformations possibly indicative of inbreeding depression have been observed in *C. e. corsicanus* (Banwell 1998; see Zachos et al. 2007 for morphological inbreeding depression in a

genetically depleted red deer population). Interestingly, there is a considerable amount of genetic differentiation between Sardinia and Corsica as revealed by the significant F_{ST} -value of 15%. The fact that the pooled *corsicanus* sample showed significant deviations from HWE at five loci—while the Corsican population yielded none at all and the Sardinian sample only two—is further evidence of this differentiation as it is best explained by the Wahlund effect—a relative excess of homozygotes in a pooled sample due to substructuring. This is in line with the non-random pattern of Corsican deer in the individual-based tree, the presence of two almost island-specific groups in the results yielded by the STRUCTURE software and the fact that only a minor fraction of *C. e. corsicanus* specimens was incorrectly assigned to their population of origin. Given that our sample sizes were 23 and 10, respectively, it is not surprising that we found alleles in either Sardinia or Corsica absent from the other one. However, the fact that a 2.3-fold increase in sample size only yielded an increase in allele number of less than 50% (30 alleles from Corsica, 44 from Sardinia) suggests that we have actually detected the majority of alleles present in *C. e. corsicanus* at the loci studied (otherwise a more linear relationship between sample size and allele number would have been expected). Thus, mutually exclusive alleles may be an additional sign of genetic differentiation. Since the introduction of Sardinian deer on Corsica was carried out only about twenty years ago (Kidjo et al. 2006), corresponding to roughly three generations in red deer, any differentiation found must have been present from the beginning due to the random sample of alleles drawn from the Sardinian population. This founder effect has resulted in incipient genetic differentiation through drift but has not led to a reduced genetic variability in the newly-founded Corsican population as was shown by the lack of significant differences in heterozygosity and allelic richness between the two islands. This may be explained by the low genetic diversity of the source population on Sardinia: a small number of founder individuals is more likely to represent a substantial proportion of the source population's gene pool if the latter only harbours comparatively small numbers of alleles.

Phylogeography of *Cervus elaphus corsicanus*

Recent studies based on mitochondrial markers (Ludt et al. 2004; Skog et al. submitted) have shed light on the general phylogeographic patterns in European red deer and identified two main glacial refugia (Iberian peninsula and the Balkans) from which Europe was recolonized in Holocene times. Palaeontological evidence, on the other hand, suggests up to five refugia including the Italian peninsula, southern France and the Carpathians (Sommer and Nadachowski 2006). In any case, the question of the origin of the red deer on Sardinia and Corsica has been answered differently by various authors. Table 4 summarizes alternative hypotheses based on phenotypic and different kinds of genetic data. The two most probable scenarios involve either mainland Italy or North-Africa, and a direct comparison of these two candidate populations has been one main aim of the present study. Both Spain and Bulgaria/the Near East have not been corroborated as probable origins of *C. e. corsicanus* in extensive studies with very large data sets of several hundred European red deer (Ludt et al. 2004; Skog et al. submitted), and the present study again shows no evidence of close nuclear genetic relationships between *C. e. hispanicus* and *C. e. corsicanus* as can be seen from the genetic distance values, the resulting trees and the relatively low proportion of *corsicanus* alleles in the Spanish population.

While all populations showed significant genetic differences (see F_{ST} -values, private alleles and the very high proportion of correct assignments of individuals to their origin), in terms of genetic similarity to *C. e. corsicanus* our data clearly yielded two groups of populations:

Table 4 Overview of the different results and hypotheses as to the origin of the Corsican red deer (*C. e. corsicanus*)

Closest to <i>C. e. corsicanus</i>	Molecular marker (or other data)	Reference
Spain, N-Africa	Phenotypic characters	Flerov (1952)
N-Africa	mtDNA (cyt <i>b</i>)	Ludt et al. (2004)
N-Africa	mtDNA (D-loop, cyt <i>b</i>)	Skog et al. (submitted)
Spain	mtDNA (D-loop)	Zachos et al. (2003), Hmwe et al. (2006a)
Bulgaria	mtDNA (RFLPs)	Hartl et al. (1995)
Mainland Italy	Microsatellites	Zachos et al. (2003), Hmwe et al. (2006a)
Mainland Italy	Microsatellites	Present study

mtDNA = mitochondrial DNA

Tunisia, Mesola and France were much closer to the Corsican red deer than Spain, Scotland or the Carpathians. Genetic similarity to Corsican red deer was expected for Tunisia and Mesola but certainly not for France. Introductions of French deer into the gene pool of *C. e. corsicanus* cannot be excluded, but it also seems possible that French and Corsican red deer would be more distinct if the number of loci analysed were higher (which would be in line with the rather low bootstrap support in the neighbour-joining tree).

Nevertheless, our study again confirmed the close relationship between *C. e. corsicanus*, Mesola and North-Africa, and among the latter two, Mesola showed lower values of genetic distance from Corsican red deer than Tunisia did. Also, allelic frequencies as shown in Fig. 2 were more similar between Mesola and *C. e. corsicanus* than between the latter and Tunisia. The present study confirms the results of Hmwe et al. (2006a), but with eight completely different nuclear loci, which adds to the credibility of the genetic similarity between red deer from mainland Italy and the Tyrrhenian islands. This is consistent with archaeozoological analyses which yielded close morphological similarities (small size, simplified antlers, Vigne 1988, 2003) between the late Holocene and modern Corsican red deer on the one hand and the small Holocene red deer from archaeological sites in Italy on the other, indicating that the latter could have given rise to the former during Holocene times. It is highly improbable, though, that the extant Tyrrhenian red deer are direct descendants of the putative Mid-Pleistocene red deer (*C. e. rossii*, Pereira 2001) because, in spite of the analysis of tens of thousands of animal bones from Corsico-Sardinian palaeontological and archaeological sites, no red deer bones have so far been found for the time between the early Holocene (ca. 11,500 cal. BP) and the mid-Holocene (5000 cal. BP or later) (Vigne et al. 1997; Vigne 1999; Costa et al. 2003). Conversely, large numbers of bones from small *C. elaphus* specimens have frequently been found in sites from the Late Neolithic (4,500 cal BP) in Sardinia (Fonzo 1987) and from Classical Antiquity in Corsica (Vigne 1988), suggesting human introductions (Vigne 1992, 1999; Masseti 1998, 2002a).

Colonisation events such as these were rather common at the time. Deer were introduced to numerous islands for hunting as early as the 11th millennium BP (Vigne 1993; Masseti 1996, 2002a, b), and a whole wave of non-flying terrestrial mammals (large and small) colonised all the large Mediterranean islands, starting at about 10,500 cal. BP in Cyprus, and more generally speaking, at the beginning of the Neolithic on various islands (Vigne and Alcover 1985; Davis 1987; Vigne 1992, 1999; Blondel and Vigne 1993; Schüle 1993; Dobson 1998; Masseti 1998).

The historical and biogeographical context, the morphological proximity between the Corsican red deer and the early Holocene deer of mainland Italy and the genetic evidence presented and reviewed in the present paper consistently corroborate that the population of *C. e. corsicanus* was probably founded on the Tyrrhenian islands with Italian mainland deer introduced by humans in the second half of the Holocene. Archaeological and historical observations suggest that these deer introductions aimed at increasing the diversity of large game species on these islands, deer hunting being valued both for meat supply and for socio-symbolic reasons (Vigne 1993).

In North Africa, most of the Late Pleistocene/Early Holocene deer remains belong to *Megacerooides algericus*, an extinct species which could not have given rise to *C. e. barbarus* (Hadjouis 1990). The presence of a Late Pleistocene *Cervus* species in the Maghreb is still questionable (Cheylan 1991), and it is much more probable that modern Barbary red deer are descendants from late Holocene introductions by humans (Kowalski and Rzebik-Kowalska 1991). The present study, having confirmed the genetic similarity of the Corsican red deer to both Mesola and North-Africa in a direct comparison, suggests that red deer from Sardinia/Corsica were introduced to North-Africa (see also Dobson 1998), thus reversing the ancestor-descendant relationship of *C. e. corsicanus* and *C. e. barbarus* which has hitherto mostly been depicted as Barbary red deer giving rise to Corsican red deer.

With all the recorded and unrecorded translocations of red deer in Europe (Hartl et al. 2003) it will probably be impossible to uncover the species' distribution history in all details. The present study, however, has at least reconciled genetic data based on different markers and palaeontological as well as archaeozoological data on the biogeographic history of *C. e. corsicanus* and its probable origin.

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