Extensive mitochondrial-DNA differentiation among European Red deer (*Cervus elaphus*) populations: implications for conservation and management

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Abstract

To investigate genetic differentiation among populations of European red deer (*Cervus elaphus*), mitochondrial(mt) DNA of a total of 70 individuals from 15 sampling sites in Western, Central, and Southeastern Europe was digested with 16 restriction enzymes. A total of 69 restriction sites allowed us to define nine haplotypes, whereby most of the samples were monomorphic for a particular haplotype. Relationships among haplotypes were largely consistent both with the geographical distribution of haplotypes among sampled populations and with relationships among samples as inferred from allozyme electrophoresis. Given the subdivision of European red deer into several distinct gene pools, mtDNA restriction profiles will serve as a powerful tool for a number of applications in conservation and management: Regarding the alteration of native gene pools by introductions of red deer from various parts of Europe, mtDNA haplotypes allow to assess the genetic impact of introductions and to trace the origin of introduced females. Even at a comparatively small geographic scale the distribution of haplotypes is helpful in detecting isolation or hybridization among populations. Finally, mtDNA restriction profiles may contribute to shed light both on the controversial systematic position and on the geographic origin of some subspecies, such as the Sardinian red deer.

Introduction

Among all extant European ungulate species the red deer (*Cervus elaphus*) is probably the one most strongly affected by anthropogenic influences on genetic population structure. Many populations are isolated because of fragmentation of the landscape (e.g. forest clearings, fenced motorways) and being kept in enclosures (cf. Kleymann 1976 a; Hartl et al. 1990 a; Ströhlein et al. 1993). Selective hunting in favour of large and branched antlers was found to result in frequency changes of allozyme marker alleles which are associated both with antler traits (Hartl et al. 1991, 1995) and with various fitness components (Pemberton et al. 1988, 1991). A potentially very important anthropogenic influence on the genetic structure of red deer populations comes from the introduction of foreign deer into autochthonous stocks. Artificial hybridization of red deer from different source populations, either in the course of restocking operations or of

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attempts to breed for larger antler size, has been a common practice in several European countries for centuries (cf. Beninde 1937, 1940). Given the variety of red deer subspecies proposed to exist in Europe on the basis of morphological characters (cf. Trense 1989), such hybridization may have caused considerable alterations of local gene pools. However, whereas many red deer populations are known to have received some introductions, the exact origin of the introducted animals is usually not recorded.

Apart from a single approach based on blood groups (Kleymann 1976 b), only allozymes and blood proteins have been used for assessing genetic differentiation among populations and presumed subspecies of European red deer so far (e.g. Bergmann 1976; Gyllensten et al. 1983; Dratch and Gyllensten 1985; Herzog 1988, 1990; Hartl et al. 1990 a, 1991, 1993 a; Herzog et al. 1991; Ströhlein et al. 1993). However, biochemical genetic differences among populations and subspecies were quantitative (variation in frequencies of ubiquitous alleles) rather than qualitative (rare, private or fixed alleles). Frequency differences of ubiquitous alleles in red deer can be ascribed to a variety of demographic factors (Ryman et al. 1981; Hartl et al. 1990 a; Herzog et al. 1991) and may to a certain extent be also due to selection (Pemberton et al. 1988, 1991; Hartl et al. 1991, 1995). Thus, in this species, electromorphs alone are not very powerful markers for resolving differentiation and for detecting introgression at the population level.

Due to a smaller effective population size for mitochondrial than for nuclear genes (Birley and Croft 1986), population specific markers should be found more likely in mitochondrial(mt) DNA than in allozymes (cf. Avise et al. 1987). Indeed, in several cases restriction site analysis of mtDNA has already been successfully applied to stock identification in the context of conservation and management of fish populations (see Avise 1994, for a review). However, in red deer, data on intraspecific mtDNA differentiation are available so far only from North American elk, where no variation among populations and subspecies could be detected (Cronin 1992). It is the aim of the present study to investigate mtDNA differentiation among European red deer populations on the basis of restriction fragment length polymorphisms. Furthermore, the distribution of mtDNA haplotypes will be evaluated as to its relevance for conservation and management of this species.

Material and methods

A total of 70 red deer from 15 sampling sites in Europe were investigated (Fig. 1). Except for the samples from Vienna and Chambord, which were drawn from populations in enclosures, all other samples are from native populations. In this context, the term 'native' refers to populations, where, at least in historical times, introductions of foreign deer are neither recorded nor likely.

MtDNA analyses

Preparation of mitochondria from frozen liver, extraction and purification of mtDNA were performed according to standard methods (HARTL et al. 1993b). MtDNA was digested by a total of 16 six-base cutting restriction endonucleases purchased from Boehringer Mannheim according to protocols provided by the supplier: ApaI, BamHI, BcII, BgIII, ClaI, DraI, EcoRI, EcoRV, HindIII, PstI, PvuII, SacI, SfuI, StyI, XbaI, XhoI. One gram of frozen liver yielded mtDNA sufficient for two to three digests. Fragments were separated electrophoretically in agarose gels containing EtBr and visualized under UV light (for details see HARTL et al. 1993b). Fragment lengths were determined using Lambda phage DNA digested with HindIII as a size standard. Restriction sites were mapped by the double-digestion method (Avise 1994). In order to make our data comparable to

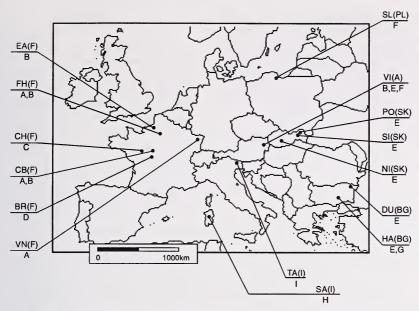


Fig. 1. Geographical distribution of sampling sites of European red deer.

those of previous studies on cervids, we attempted to adjust the starting site for mapping by comparing our data with those presented on North American elk by Cronin (1991).

Allozyme analyses

For assessing correspondence of major groups of European red deer as revealed both by mtDNA and allozyme analyses, we used those samples, where numbers of available individuals were sufficient for obtaining reasonable estimates of allozyme allelic frequencies (n > 25, except for DU, n = 13, and for SL, n = 10). Data are based on 43 presumptive loci screened by HARTL et al. (1993 a) in samples from SI, PO, DU, HA, and by HARTL et al. (unpubl.) in samples from Poland (SL) and France.

Statistics

Relationships among haplotypes were inferred by the following methods: Based on restriction sites (Tab. 1) we calculated the mean number of base substitutions per nucleotide (p) using formulas 10 and 8 in Nei and Li (1979). To display relationships among mtDNA haplotypes, the p-values were used to generate an unrooted tree by means of the FITCH option in Felsenstein's PHYLIP-package (Felsenstein 1988). This procedure was preferred to standard cluster analysis because of the possibility of differences in evolutionary rates among major mtDNA lineages. Based on Nei's (1978) unbiased genetic distance, the same method was used for inferring genetic relationships among samples as revealed by allozyme allelic frequencies. Cladistic analysis of mtDNA data was performed by using restriction sites as characters and calculating Wagner parsimony trees by means of the MIX option (PHYLIP). Clades remaining stable in various equally parsimonious trees were separated from ambiguous ones by calculating a majority rule consensus tree (CONSENSE option in PHYLIP).

Table 1. Numerical restriction maps of 9 mtDNA haplotypes (A–I) in European red deer. 1 (0) = restriction site present (absent). Among the 16 restriction endonucleases applied, only XhoI had no cutting site. Based on the comparison of cutting sites of restriction enzymes used in both studies, the starting point for mapping corresponds to that of Cronin (1991).

Enzyme Position	I
ApaI 0.1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	I
3.6 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
BamHI 0.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
BamHI 0.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
BamHI 0.4 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 </td <td>1</td>	1
BcII	0
BelI 0.1 0 1 0 0 1 1 0 0 0 1 1 1 0 0 0 2.9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
BcII 0.1 0 1 0 0 1 1 0 0 0 1 1 1 0 0 0 2.9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0
2.9	1
Here Here Here Here Here Here Here Here	1
5.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 <td>1</td>	1
6.3 0 0 0 0 0 0 0 1 0 7.7 1 1 1 1 1 1 1 1 1 1 11.2 1 1 1 1 1 1 1 1 1 11.9 1 1 1 1 1 1 1 1 1 1 11.9 1 1 1 1 1 1 1 1 1 1 15.8 1 1 1 1 1 1 1 1 0 1 ClaI 1.2 1 1 1 1 1 1 1 1 1 1 10.5 1 1 1 1 1 1 1 1 1 1 DraI 0.5 1 1 1 1 1 1 1 1 1 1.1 1 1 1 1 1 1 1.9 1 1 1 1 1 1 1 1 1	1
7.7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
BgIII 0.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0
BgIII 0.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
BgIII 0.4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
ClaI 1.2 1 1 1 1 1 0 1 ClaI 1.2 1 1 1 1 1 1 1 1 1 10.5 1 1 1 1 1 1 1 1 1 1 DraI 0.5 1 1 1 1 1 1 1 1 1 1 1.1 1 1 1 1 1 1 1 1	1
ClaI 1.2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
DraI 0.5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
DraI 0.5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
1.1 1 1 1 1 1 1 1 1 1.9 1 1 1 1 1 1 1 1	1
1.9 1 1 1 1 1 1 1 1	1
	1
25 1 1 1 1 1 1 1 1	1
3.5 1 1 1 1 1 1 1 1	1
5.6 1 1 1 1 1 0 1 1	1
11.3 1 1 1 1 1 1 1 1	1
12.5 1 1 1 1 1 1 1 1	1
13.6 1 1 1 1 1 1 1 1	1
16.2 1 1 1 1 1 1 1 1	1
EcoRI 9.3 1 1 1 1 1 1 1 1	1
12.1 1 1 1 1 0 1 0 0	0
12.4 0 0 0 0 1 0 0 0	1
15.7 1 1 1 1 1 1 1 1	1
EcoRV 2.9 1 1 1 1 1 1 1 1	1
5.6 1 1 1 1 1 1 1	1
12.8 1 1 1 1 1 1 1 1	1
13.6 1 1 1 1 1 1 1	1
15.8 1 1 1 1 1 0 0	1
HindIII 6.0 1 1 1 1 1 1 1 1	1
7.8 1 1 1 1 1 1 1	1
12.0 1 1 1 1 1 1 1 1	1
PstI 8.8 1 1 1 1 1 1 1 1	1
9.5 1 1 1 1 1 1 1 1	1
PvuII 9.2 1 1 1 1 1 1 1 1 1	1
9.9 1 1 1 1 1 1 1 1	1
SacI 7.5 1 1 1 1 1 1 1 1	1
9.1 1 1 1 1 1 1 1 1	
	1

Results

Cutting red deer mtDNA with 16 restriction enzymes yielded a total of 69 restriction sites, representing approximately 400 bp of mtDNA sequence or 2.4% of the 16,600 bp genome generally found in cervids (cf. Cronin 1991). The restriction site data allowed us to define nine haplotypes (A – I, Tab. 1). The distribution of haplotypes among the various red deer samples studied is shown in table 2. Most single samples or groups of geographically proximate samples were monomorphic for a unique haplotype and, thus, an analysis of relationships among haplotypes corresponded to a certain extent to that of genetic relationships among presumptive populations. Pairwise values of nucleotide divergence (p) among haplotypes are presented in table 3, overall phenetic relationships among haplotypes are shown in a FITCH-tree in figure 2. Relationships among haplotypes as revealed by a character state approach (WAGNER parsimony) are shown in a majority rule consensus tree (Fig. 3), calculated from the 24 most parsimonious solutions found in resolving the restriction site matrix in table 1. Both trees yielded essentially the same result: a major separation of haplotypes A-F from haplotypes E and I, and from haplotypes G and H, the latter two groups being also quite distinct from one another.

The major tree pattern obtained from allozyme data was fairly similar to that based on the geographic distribution of mtDNA haplotypes (see Fig. 4). The samples with haplotype E were generally well separated from those with haplotypes A, C, and D. Of all samples with haplotype E, the one polymorphic for haplotypes E and G (HA) was most separated from samples with haplotypes A-D. The overall rather intermediate position of samples monomorphic for haplotypes B and F (Fig. 4) conformed with relationships among haplotypes as shown in figures 2 and 3. There B and F held a linking position between haplotypes A, C, D on the one hand, and haplotypes E and I on the other.

Table 1. (continued)

Enzyme	Position	Haplotypes								
		A	В	С	D	Е	F	G	Н	I
SfuI	3.4	1	1	1	1	1	1	1	1	1
	8.2	1	1	1	1	1	1	1	1	1
	9.9	1	1	1	1	0	1	1	1	0
	11.1	1	1	1	1	1	1	1	1	1
	12.2	1	1	1	0	1	1	1	1	1
	13.5	1	1	1	1	1	1	1	1	1
	14.8	1	1	1	1	1	1	1	1	1
StyI	2.2	1	1	1	1	1	1	1	1	1
	2.6	1	1	1	1	1	1	1	1	0
	3.0	1	1	1	1	1	1	1	1	1
	3.5	1	1	1	1	1	1	1	1	1
	6.8	1	1	1	1	1	1	1	1	1
	8.4	0	0	0	0	0	0	1	1	0
	9.5	1	1	1	1	1	1	1	1	1
	10.3	1	1	1	1	1	1	1	1	1
	10.9	1	1	1	1	1	1	1	1	1
	12.6	1	1	1	1	1	1	1	1	1
	15.3	0	0	1	0	0	0	0	0	0
XbaI	1.3	1	1	1	1	1	1	1	1	1
	4.3	1	1	1	1	1	1	1	1	1
	5.8	1	1	1	1	1	1	1	1	1
	14.7	1	1	1	1	1	1	1	1	1
	15.5	1	1	1	1	1	1	1	1	1

Discussion

In the present study, covering red deer populations from Western, Central, and Southeastern Europe, we detected considerable mtDNA differentiation among local samples. Both the exclusive occurrence of particular mtDNA haplotypes in single samples or in groups of geographically proximate samples, and the extent of nucleotide divergence among haplotypes (p = up to 0.4%) were unexpected in the light of previous results. The first study on intraspecific mtDNA variation in red deer was conducted by Cronin (1992), who examined four subspecies of North American elk across a sampling area comparable to ours. Except for one case, where the mtDNA of one out of 10 individuals deviated at one out of 44 restriction sites, all subspecies were found monomorphic for the same haplotype. By comparing mtDNA differentiation in elk with that in other cervid species Cronin (1992) proposed that the lack of significant variation among elk populations and subspecies may reflect founder effects

following colonization of North America south of Alaska during the late Pleistocene. Our results support Cronin's hypothesis in that they show that red deer mtDNA is not generally less variable among populations than in the other species of Cervidae studied so far.

Table 2. Sampling sites of red deer in Europe (code, name, country), and distribution of mtDNA haplotypes (A – I) among samples.

Sampling site	Haplotypes								
	A	В	С	D	Е	F	G	Н	I
VN Vosges du Nord (F)	6	_	_	_	_	_	_	_	_
EA Eany (F)	_	6	_	_	_	_	_	_	_
CH Champchevrier (F)	_	_	5	_	_	_	_	_	_
BR Brouard (F)	_	_	_	5	_	_	_	_	_
FH Forêt de Halatte (F)	2	4	_	_	_	_	_	_	_
CB Chambord (F)	1	3	_	_	_	_	_	_	_
PO Polana (SK)	_	_	_	_	5	_	_	_	_
SI Sitno (SK)	_	_	-	_	2	_	_	_	_
NI Nitra (SK)	_	_	_	_	2	_	_	_	_
SL Slowinski (PL)	_	_	_	_	_	7	_	_	_
DU Dulovo (BG)	_	_	_	_	4	_	_	_	_
HA Haskovo (BG)	_	_	_	_	3	_	2	_	_
SA Sardinia (I)	_	_	_	_	_	_	_	3	_
TA Tarvis (I)	-	_	_	_	_	_	_	_	4
VI Vienna (A)	-	2	-	-	2	2	-	-	-

Table 3. Sequence divergence (p, in per cent) among the nine mtDNA haplotypes detected in Eur-
opean red deer.

	A	В	C	D	Е	F	G	Н	I
Α	_								
В	0.04	-							
C	0.04	0.09	_						
D	0.04	0.09	0.09	-					
E	0.18	0.14	0.23	0.23	-				
F	0.09	0.04	0.13	0.14	0.18	-			
G	0.28	0.32	0.32	0.33	0.38	0.37	_		
Н	0.23	0.27	0.27	0.28	0.33	0.32	0.14	_	
I	0.23	0.18	0.28	0.28	0.05	0.23	0.43	0.38	-

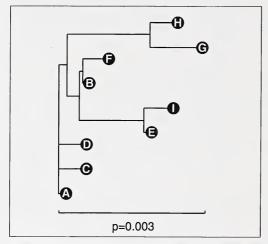


Fig. 2. Unrooted tree showing phenetic relationships among mtDNA haplotypes A – I (p, NEI and LI 1979/FITCH-tree, PHYLIP).

Because of the lack of data on closely related outgroup species and on red deer from several important nuclei in Southwestern, Northern, and Eastern Europe our data presently are too scarce to provide a comprehensive pattern of phylogenetic relationships in European red deer. However, the distribution of mtDNA haplotypes within and among samples allows several conclusions relevant for conservation and management of this species:

In our data we found a major distinction among three mtDNA lineages (Figs. 2, 3). The "northwestern (NW) lineage" comprised several haplotypes (A, B, C, D, F) found in red deer samples from France and Poland (Tab. 2, Fig. 1). The "eastern (EA) lineage" comprised two haplotypes found in

samples from Slovakia and Bulgaria (type E), and in the sample from Northern Italy (type I). The "southeastern (SE) lineage" comprised two haplotypes found in one sample from Bulgaria (type G) and in the sample from Sardinia (type H).

We are aware that evaluating subspecies status by considering a particular magnitude of genetic distance obtained from just one marker system a threshold can be seriously misleading (cf. Hartl et al. 1990 b; Stüwe et al. 1992; Cronin 1992, 1993). Moreover, especially in mtDNA, separation of lineages may not always be consistent with separation of populations or subspecies (Avise 1989; Cronin 1993). However, with some exceptions discussed below (Vienna, Sardinia), the geographical distribution of haplotypes of the three major lineages may correspond to the distribution of red deer subspecies as proposed by Groves and Grubb (1987) on the basis of morphological criteria: The NW-lineage would represent *C. e. elaphus* (synonyms: *C. e. hippelaphus, C. e. germanicus*, Wagenknecht 1983; Gyllensten et al. 1983), and the EA-lineage *C. e. montanus*. The SE-lineage (especially type G found in sample HA from Bulgaria) may be speculated to stem from *C. e. maral*, which is described for Turkey, the Caucasus and Kurdistan, but possibly ranges also to Southeastern Europe (Groves and Grubb 1987). Separation

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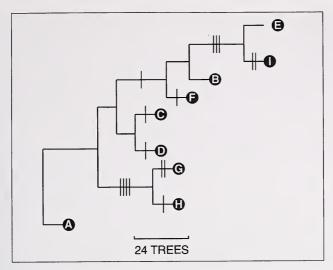


Fig. 3. Majority rule consensus tree showing cladistic relationships among haplotypes. The tree is based on 24 equally parsimonious trees obtained from Wagner maximum-parsimony analysis (MIX option, PHYLIP) of presence or absence of 69 restriction sites (Tab. 1). Both the parsimony trees and the consensus tree are basically unrooted trees, which were arbitrarily rooted at haplotype A. Distances between nodes refer to the number of trees in which the respective clusters were found. The vertical bars indicate the number of restriction site gains and losses (15 in total) for one of the parsimony trees, which was topologically largely identical to the consensus tree.

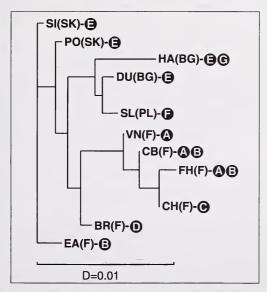


Fig. 4. Unrooted tree showing genetic relationships among 11 samples used in the present study as revealed by electrophoretic analysis of 43 allozyme loci (D, Nei 1978/FITCH-tree, PHYLIP). Haplotypes found in the respective samples are indicated by white letters following the sampling site/country code (data for SI, PO, HA, and DU are from HARTL et al. 1993 a, data for SL and the French samples are unpublished).

among the western (NW) and the eastern (EA, SE) mtDNA lineages as well as differences between eastern lineages (EA – SE) may be attributed to different glacial refugia (cf. BENINDE 1937; GEIST 1971). Indeed, when divergence times (t) are calculated from nucleotide divergence, assuming that p = 2% roughly corresponds to t = 1 million years (Wilson et al. 1985), separation between the NW and each of the two eastern (EA, SA) types dates back to the Riss-Würm interglacial (ranging from about 130,000 to 70,000 years before present, HAQ and VAN EYSINGA 1987). As far as allozyme data are available, they confirm the major distinction between the northwestern and the eastern groups (especially EA) of red deer suggested by the distribution of mt DNA haplotypes (Fig. 4).

Massive changes in the genetic structure of native populations as brought about by artificial hybridization may result in the distortion of well adapted gene pools (Templeton et al. 1986). The genetic impact of introductions on red deer populations is one of the oldest and still unsettled questions in the management of European red deer (cf. Beninde 1940). Given the local restriction of major and minor lineages, mtDNA restriction profiles may serve as a powerful tool for assessing the contribution of introduced animals to autochthonous gene pools as well as for tracing their origin.

For example, the Vienna sample came from a breeding population kept in an enclosure at the Forschungsinstitut für Wildtierkunde und Ökologie for research purpose. Founder individuals of that population originated from several hunting areas in Austria. Altogether, three haplotypes (B, E, and F) were detected, which may be due to the origin of the deer from different parts of Austria. Alternatively, the polymorphism may reflect a past introduction of foreign deer into one or more of the source populations. Once a large scale screening for the distribution of mtDNA variants among as many autochthonous populations as possible is completed, questions as to the approximate geographic origin of the respective founder animals of mixed populations should readily to be answered.

Given the increasing fragmentation of habitats by fenced motorways, concreted channels, settlements, and forest clearings, formerly large and homogeneous populations of large game animals are increasingly fragmented and isolated. Within such small isolates, losses of genetic variation and, hence, of adaptability are to be expected (see HARTL and PUCEK 1994). So far, isolation of populations and losses of genetic variability in the red deer have been assessed using only protein electrophoretic data (e.g. Bergmann 1976; HARTL et al. 1990 a, 1991; HERZOG et al. 1991; STRÖHLEIN et al. 1993).

Due to its haploid and uniparental mode of inheritance in mammals, effective population size (Ne) for mtDNA amounts to only 1/4 of that for nuclear DNA (BIRLEY and CROFT 1986). Given the presence of polymorphism in the original population, a subdivision of that population into small isolates should be more readily detectable using mtDNA haplotypes than nuclear markers. Four out of our six red deer samples from France were found monomorphic for different haplotypes (A, B, C, D, see Tab. 2, Fig. 1), and two were polymorphic for haplotypes A and B. It is likely that the monomorphic samples represent four populations, which are genetically isolated from one another. Of the two polymorphic samples, one came from a population kept in an enclosure (CB), which is known to contain French red deer of different origin. The remaining sample (FH) may either represent a population in more ancestral condition (i.e. polymorphic) or a hybrid population of pure stocks monomorphic for type A and B, respectively. Nevertheless, although further studies are necessary for investigating the French situation in more detail, our data may be sufficient for demonstrating the power of mtDNA restriction profiles in resolving migration patterns among European red deer stocks even within a restricted geographic area. The monomorphism for haplotype E in Slovakian samples is probably the result of genetic bottlenecks. Slovakian red deer was almost completely exterminated by the end of the 18th century, and populations were restocked with red deer from adjacent geographic areas in the east and in the south (P. Hell, pers. comm.).

Given the coincidence of relationships among haplotypes and the geographic location of sampling sites where they were found (recall that haplotype F was intermediate between the NW and the EA lineage, Figs. 1, 2), the geographically very separate position of populations showing the closely related haplotypes G (HA, Fig. 1) and H (SA) is somewhat surprising. The relationship among haplotypes in a Bulgarian and the Sardinian sample may be due to a common inheritance from a remote ancestor, which cannot be tested without increasing considerably the range of populations sampled. Generally, the origin of Sardinian red deer (*C. e. corsicanus*) is still a matter of debate (Groves and Grubb 1987). Based on phenotypic resemblance, Flerov (1952) considered the deer of Sardinia, North Africa, and Southern Spain as belonging to the same subspecies. However, small body size and simple antler shape may be the parallel outcome of equally poor ecological conditions (cf. Beninde 1937; Geist 1986).

According to paleontological results, red deer had been absent from Sardinia until about 8,000 before present. The oldest red deer fossils were found in the Corbeddu Caves of the Old Neolithic together with those of mouflon (Ovis ammon musimon), a species that had also been absent from the island before (SANGES 1987). This date coincides with the advent of the Early Mediterranean Neolithic culture on the island, characterized by the production of Cardium ceramics (Masseti and Vianello 1991; Masseti 1993). There is already clear evidence that the mouflon was introduced to Sardinia and to other islands of the Western Mediterranean by human settlers from the Near East (GEDDES 1985; VIGNE 1992). The contemporary appearance of red deer on Sardinia and the similarity of its mtDNA haplotype with that found in a sample from Southern Bulgaria, possibly representing C. e. maral (see Groves and Grubb 1987), may suggest that the origin of Sardinian red deer is the same as in the mouflon. Compared with red deer from Southeastern Europe, the exceptionally small body size presently found in Sardinian red deer (Beninde 1937) may be attributed to a decline of body size as reported for many island forms (SOONDAR 1977; DAMUTH 1993). Further genetic studies, especially on red deer populations from Turkey and Southern Spain, are required for testing this hypothesis.

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Zusammenfassung

Ausgeprägte Mitochondrien-DNA-Differenzierung zwischen europäischen Rothirschpopulationen (Cervus elaphus L.) und deren Bedeutung für den Artenschutz und die Wildbewirtschaftung

Um die genetische Differenzierung zwischen europäischen Rothirschpopulationen (*Cervus elaphus*) zu untersuchen, wurde die mitochondriale(mt) DNA von insgesamt 70 Individuen aus 15 Probengebieten in West-, Mittel- und Südosteuropa mittels 16 Restriktionsendonukleasen verdaut. Eine Gesamtzahl von 69 gewonnenen Schnittstellen erlaubte die Definition von 9 Haplotypen, wobei sich die meisten Stichproben für jeweils den einen oder anderen Haplotyp als monomorph erwiesen. Die phylogenetischen Beziehungen zwischen den Haplotypen stimmten weitgehend mit deren geographischer Verteilung und mit den aus Alloenzymdaten abgeleiteten genealogischen Beziehungen zwischen den untersuchten Beständen überein. Nachdem sich europäische Rothirschbestände hinsichtlich des Vor-

kommens von mtDNA-Typen deutlich voneinander unterscheiden, verspricht dieses genetische Markersystem für eine Reihe von Fragestellungen im Rahmen des Artenschutzes und der Wildbewirtschaftung in der Zukunft große Bedeutung zu erlangen: Hinsichtlich einer Veränderung autochthoner Genpools durch Einbürgerungen standortfremden Rotwildes erlaubt die mtDNA sowohl eine Abschätzung des Fortpflanzungserfolges eingebürgerter weiblicher Individuen als auch eine großräumige Bestimmung deren Herkunft. Die mtDNA erweist sich aber auch kleinräumig zur Feststellung der Isolation oder der Hybridisierung von Populationen als wertvoll. Schließlich können, wie im Falle des Sardinischen Rothirsches, Restriktionsprofile der mtDNA auch zur Abklärung der systematischen Stellung von Unterarten sowie deren geographischer Herkunft beitragen.

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