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Biochemical genetic description of German and Swiss populations of red deer *Cervus elaphus*

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A total of 228 red deer *Cervus elaphus* Linnaeus, 1758 from 5 sampling sites in Germany and 2 sampling sites in Switzerland were analysed for allozyme variability by means of various electrophoretic techniques. Based on 26 presumptive structural loci scored, the proportion of polymorphic loci (*P*) ranged from 3.8% to 11.5%, and average heterozygosity (*H*) varied between 0.8% and 2%. These values are among the lowest ones as yet detected in population genetic studies of red deer. A correlation between genetic and geographic distances could be demonstrated and significant allele frequency differences were observed, especially between the German and the Swiss populations examined. Levels of relative genetic differentiation (G_{ST}) among populations in Germany and Switzerland were low and amounted to 3.9% and 1.5%, respectively. When compared with all other German samples, a private allele at the *Mpi-1* locus and remarkably different allelic frequencies at the *Sod-2* locus were detected in the game preserve Reinhardswald. These findings were interpreted to result from a release of Hungarian and Hungarian × Yugoslavian red deer in the 1980s.

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Key words: Cervus elaphus, electrophoresis, genetic variability, genetic distances, game management

Introduction

In Germany the habitat of red deer *Cervus elaphus* Linnaeus, 1758 is increasingly fragmented. Apart from barriers associated with the cultivation of the landscape also hunting directions prevent migration among deer regions very effectively: except for stags with crown antlers, all red deer found outside of these regions have to be shot. Thus, gene flow is almost impossible and the whole red deer population is divided into many reproductively isolated subpopulations. Small, isolated demes are particularly exposed to losses of allelic variation by genetic drift and are known to be possible candidates for inbreeding depression (e. g. Urbston *et al.* 1976, Johns *et al.* 1977, O'Brien *et al.* 1983).

In the present paper, genetic diversity within and among red deer populations of Germany (Hessen) and Switzerland (regions north and south of Walensee) is evaluated. The management implications emerging from our data are discussed.

Material and methods

Liver samples of 163 red deer were collected during the years 1989 to 1992 from the following Hessian regions (abbreviations in parentheses): Reinhardswald (RW), Werra-Fulda (W-F), Spessart (S), Dill-Bergland (D-B), and Rothaargebirge (RG). Liver samples of 65 specimens were collected from the red deer regions north (N-S) and south (S-S) of the Walensee, Switzerland (the geographic location of sampling areas is displayed in Figs 1 and 2).

In order to achieve the best resolution of the respective allozymes, the following electrophoretic techniques were applied (abbreviations in parentheses): agarose gel electrophoresis (AGE), cellulose acetate electrophoresis (CAFE), and ultrathin-layer isoelectric focusing in 200 m polyacrylamide gels (UDIEF). The following enzyme systems were screened (abbreviation, E. C. number and electrophoretic technique used are given in parentheses): alcohol dehydrogenase (ADH, 1.1.1.1, AGE), sorbitol dehydrogenase (SDH, 1.1.1.14, UDIEF), lactate dehydrogenase (LDH, 1.1.1.27, AGE), malate dehydrogenase (MDH, 1.1.1.37, AGE), malic enzyme (ME, 1.1.1.40, UDIEF), 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44, AGE), glucose dehydrogenase (GDH, 1.1.1.47, AGE), glucose-6--phosphate dehydrogenase (G-6-PD, 1.1.1.49, AGE), glutamate dehydrogenase (GLUD, 1.4.1.3, AGE), superoxide dismutase (SOD, 1.15.1.1, UDIEF), hexokinase (HK, 2.7.1.1, AGE), phosphoglucomutase (PGM, 2.7.5. 1, AGE), peptidases A and B (PEP-A, PEP-B, 3.4.11, CAFE), mannose phosphate isomerase (MPI, 5.3.1.8, UDIEF), and phosphohexose isomerase (PHI, 5.3.1.9, UDIEF). For specific electrophoretic procedures and staining recipes see Ströhlein (1993).

Enzyme loci and alleles were designated as follows: electrophoretic bands corresponding to the products of different loci or alleles were continuously numbered from the cathodal to the anodal end of the gel.

To test for the homogeneity of allelic frequencies the method of Nei and Roychoudhury (1974) was used. Observed genotypes were examined for an agreement with the distribution of genotypes expected in the case of Hardy-Weinberg equilibrium using the *G*-test (Sokal and Rohlf 1969). As levels of significance we chose $p < 0.05$ (*) and $p < 0.01$ (**). Genetic distances were calculated according to Gregorius (1974) and the dendrogram was constructed using the UPGMA-algorithm (Sneath and Sokal 1973). Relative genetic differentiation was assessed using Nei's (1973) *G*-statistic. The average number of alleles per locus (A), the proportion of polymorphic loci (*P*, 99% criterion), average heterozygosity (*H*), and G_{ST} were calculated using a Turbo Pascal (4.0) program.

Results

Three out of 26 putative loci were found to be polymorphic. These are coding for the dimeric enzymes superoxide dismutase (SOD) and phosphohexose isomerase (PHI) with two alleles each, and the monomeric enzyme mannosephosphate isomerase (MPI) with three alleles (Fig. 2).

The frequencies of the most common allele at each polymorphic locus are shown in Table 1. Except for Reinhardswald, the frequencies of the allele $Sod\text{-}2^{100}$ are very similar in the German populations. The rare allele $Mpi\text{-}1^{100}$ was found only in the sample of the Reinhardswald, whereas the populations from Rothaargebirge and Dill-Bergland were monomorphic for $Mpi\text{-}1^{165}$. At the *Phi* locus, the rare allele "*110*" was observed only in the populations Rothaargebirge, Spessart, and the region south of the Walensee.

Overall allelic frequencies were homogeneous among the populations Reinhardswald, Werra-Fulda, Rothaargebirge, Spessart, and the population of the

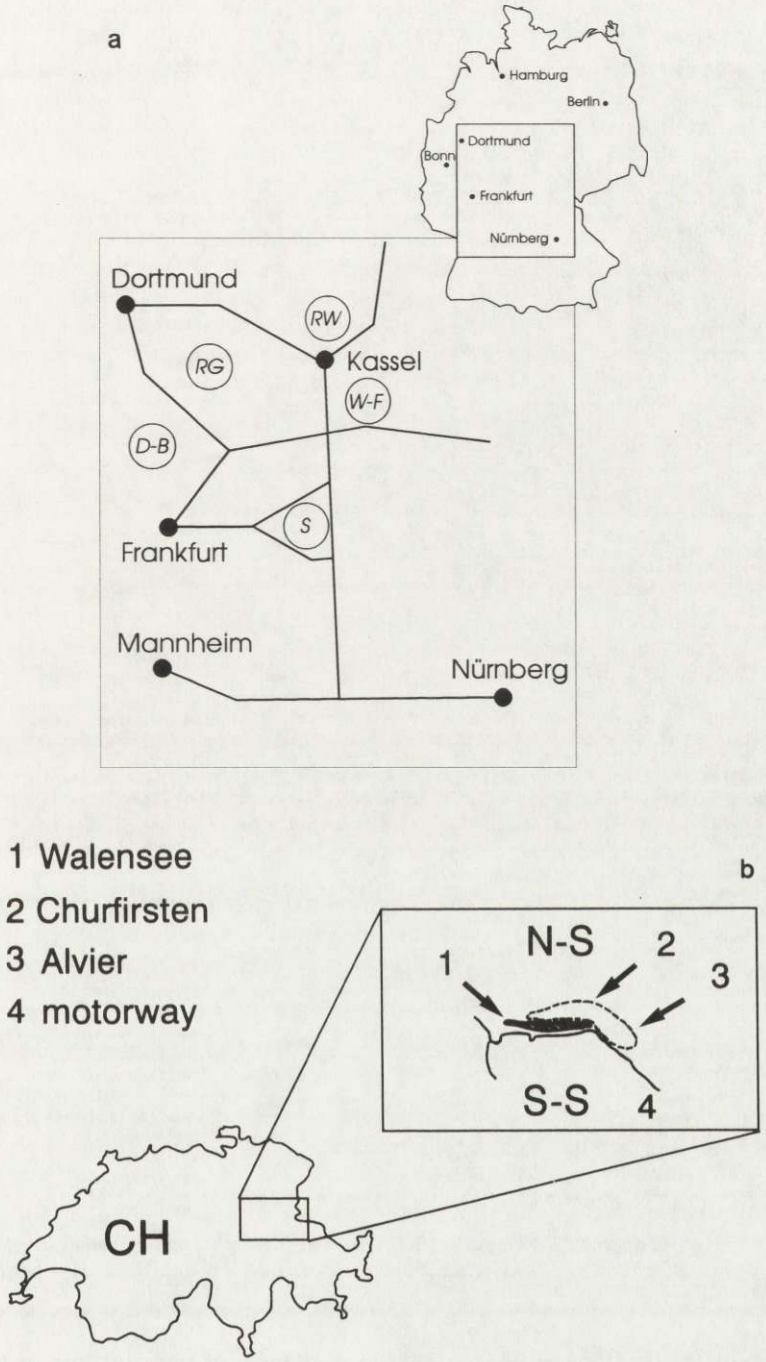

Fig. 1. Geographic location of sampling areas of red deer in Germany (a) (RW – Reinhardswald, W-F – Werra-Fulda, S – Spessart, D-B – Dill-Bergland, RG – Rothaargebirge), and in Switzerland (b) (N-S – region north of Walensee, S-S – region south of Walensee).

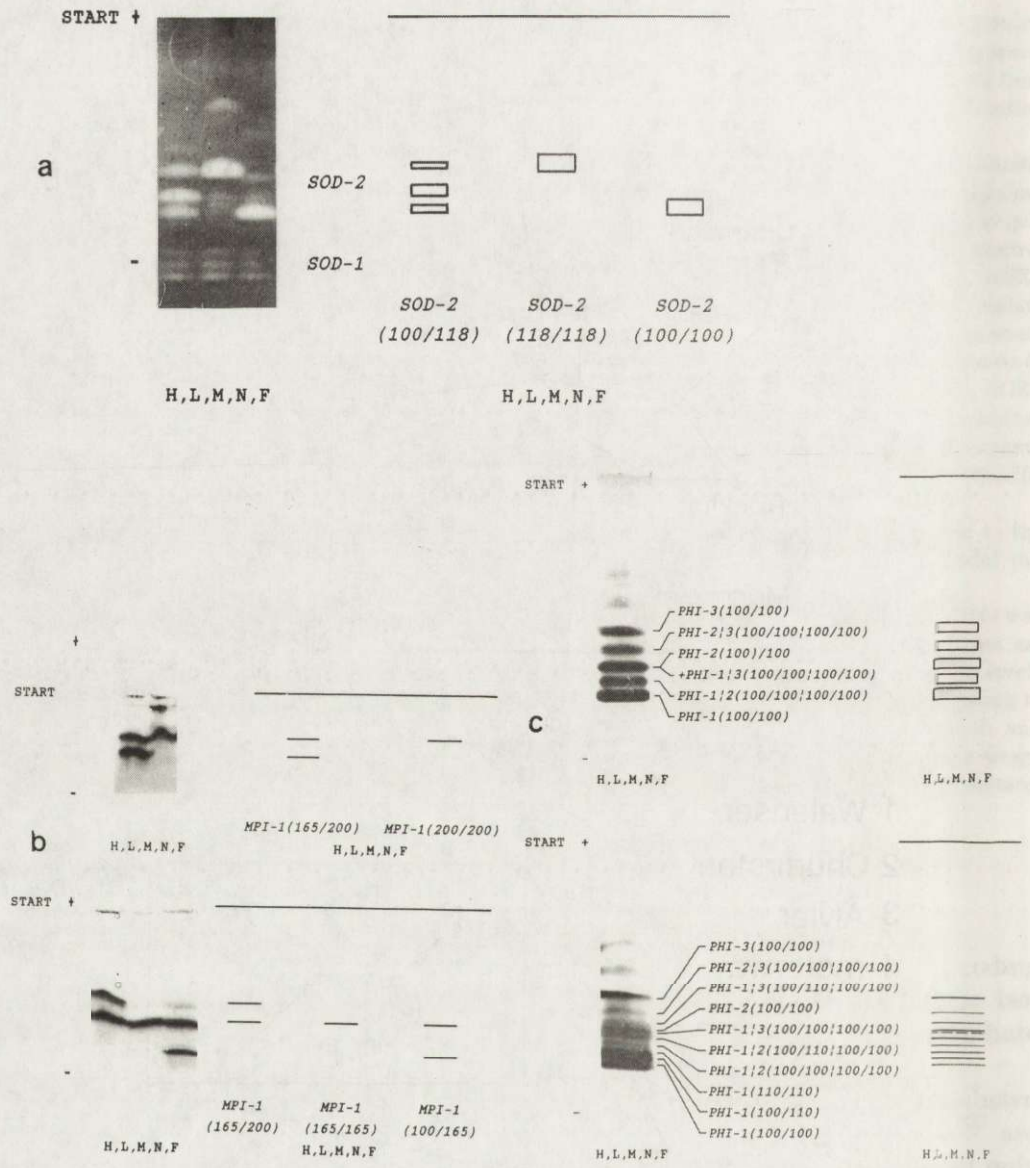

Fig. 2. Band-patterns of superoxide dismutase (SOD) (a), mannose phosphase isomerase (MPI (b), and phosphohexose isomerase (PHI) (c), in German and Swiss red deer populations. H – heart, L – liver, M – muscle, N – kidney, F – fetus.

region north of the Walensee (Table 2). Among German populations, a difference in allelic frequencies became apparent only between the Reinhardswald and the Rothaargebirge. This result is due to the preponderance of *Mpi-1*^{165} in the

Table 1. Frequencies of the most common alleles at the loci polymorphic in German and Swiss red deer. Sample sizes in parentheses. * – frequency of the private allele $Mpi\text{-}1^{100}$.

Region	$Sod\text{-}2^{118}$	$Mpi\text{-}1^{165}$	$Phi\text{-}1^{100}$
RW – Reinhardswald	0.873 (51)	0.781 (48) 0.083 (48)*	1.0 (49)
W-F – Werra-Fulda	0.911 (28)	0.865 (26)	1.0 (26)
RG – Rothaargebirge	0.922 (32)	1.0 (32)	0.969 (32)
D-B – Dill-Bergland	0.950 (10)	1.0 (4)	1.0 (10)
S – Spessart	0.917 (42)	0.821 (39)	0.966 (44)
N-S – north of the Walensee	0.694 (31)	0.952 (31)	1.0 (31)
S-S – south of the Walensee	0.809 (34)	0.909 (33)	0.985 (34)

Table 2. Test for homogeneity of allelic frequencies (χ^2-test according to Nei and Roychoudhury 1974). * – $p < 0.05$, ** – $p < 0.01$ (degrees of freedom in parentheses).

Region	S-S	N-S	S	D-B	RG	W-F	RW
RW	4.91(4)	8.50*(3)	5.99(4)	0.49(1)	10.1*(4)	2.66(3)	–
W-F	1.94(3)	5.50*(2)	1.19(3)	0.16(1)	5.45(3)	–	
RG	5.05(3)	7.90*(3)	5.45(3)	0.41(2)	–		
D-B	1.30(2)	2.70(1)	0.47(2)	–			
S	3.43(3)	9.90**(3)	–				
N-S	2.07(3)	–					
S-S	–						

Table 3. Allelic frequencies at the loci polymorphic in German and Swiss red deer.

Locus	*Sod-2*		*Mpi-1*			*Phi-1*	
Allele	*118*	*100*	*200*	*165*	*100*	*100*	*110*
Germany	0.904	0.096	0.124	0.850	0.026	0.985	0.015
Switzerland	0.787	0.213	0.070	0.930	–	0.993	0.007

Table 4. Proportion of polymorphic loci (P, 99% criterion) and average heterozygosity (H) in the red deer populations studied. P and H are calculated over 26 presumptive structural loci.

Region	RW	W-F	RG	D-B	S	N-S	S-S
P	0.077	0.077	0.077	0.038	0.115	0.077	0.115
H	0.023	0.015	0.008	0.004	0.020	0.020	0.019

Rothaargebirge and the exclusive occurrence of *Mpi-1*^{100} in the Reinhardswald. At all polymorphic loci, allelic frequencies averaged over all German and Swiss populations, respectively, were significantly different ($p < 0.05$, Table 3).

Indices of genetic variability (P, H), calculated over 26 loci examined, are given in Table 4. The average P for Germany and Switzerland was 7.7% and 9.6%, respectively. The weighted heterozygosity was 1.7% for Germany and 2% for Switzerland. The average number of alleles per locus amounted to 1.15% in both groups of samples.

A dendrogram displaying genetic similarity among the German and Swiss red deer populations studied is shown in Fig. 3. There was a significant correlation between genetic and geographic distances ($r = 0.62$, $p < 0.05$). Estimates of gene diversity among populations (G_{ST}) proved to be comparatively low. G_{ST} was 3.9% among German populations, 1.5% among Swiss populations, and 1.4% between Germany and Switzerland.

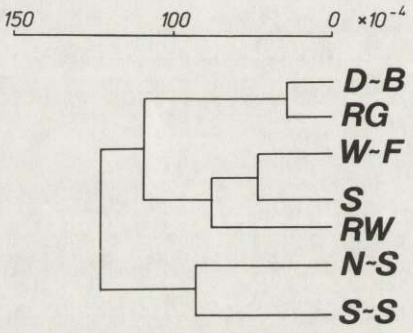

Fig. 3. Genetic relationships among German and Swiss red deer populations. The dendrogram (UPGMA, Sneath and Sokal 1969) was constructed using genetic distances according to Gregorius (1974) and is based on 26 loci.

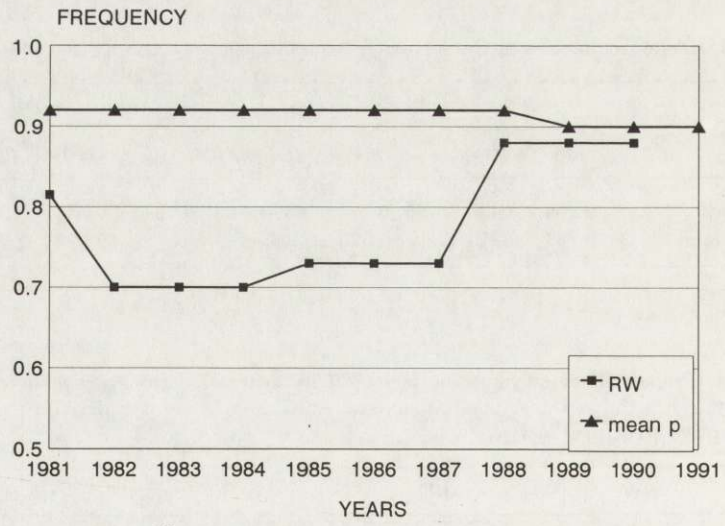

Fig. 4. Frequencies of *Sod-2*^{118} in the red deer population from Reinhardswald and mean frequencies of *Sod-2*^{118} in the other German populations, inferred from specimens of different age groups for the years 1981 – 1991.

Discussion

Except for the corresponding values in Norvegian populations (Gyllensten *et al.* 1983), the proportion of polymorphic loci and average heterozygosity found in the present study are the lowest as yet detected in red deer.

The low average heterozygosity in the Swiss populations may be the result of the history of red deer in this country. Until about 100 years ago red deer was absent. Later an immigration of red deer from Vorarlberg (Austria) to Graubünden (Switzerland) took place, and in 1934 a total of 270 individuals could already be culled in the Swiss Cantons Graubünden and St. Gallen (Wagenknecht 1981). Nevertheless, the immigration rate of red deer from the eastern regions and, thus, the influx of allelic variation was and is probably low. The existing red deer population had established with only a few individuals with high genetic similarity and possible recent immigrants obviously did not increase its allelic potential.

The low *H*-values in the German populations are due to high frequencies of the most common alleles, especially at *Sod-2* and *Phi-1*. The risk of an ultimate fixation of these alleles is given. This tendency towards fixation is illustrated by the distribution of the allele *Mpi-1*^{165}. It is the only *Mpi-1* allele occurring in the populations Rothaargebirge and Dill-Bergland, whereas in the populations Werra--Fulda and Spessart its frequency increased from 80% to 85.4% within ten years (1981 – 1991). At *Phi-1*, the allele "*100*" is predominant in the populations Reinhardswald, Werra-Fulda, and Dill-Bergland. In contrast, the rare allele *Phi-1*^{110} could be detected only in older specimens and only in heterozygous condition, suggesting that its frequency is being in reduction.

The frequency of *Sod-2*^{118} in the Reinhardswald is remarkably different from that in the other German populations. In order to interpret this result it is necessary to subdivide the sample into age groups using also data from a previous investigation (Ströhlein *et al.* 1991). Before 1981 the frequency was 81%. This frequency decreased to 73% in the age group born in 1983 and rose to 89% in 1989 (Fig. 4). A comparison of allelic frequencies of different age groups with the respective average allelic frequencies found in the other German populations shows a significant deviation of the age groups born in 1983 ($p < 0.01$) and 1986 ($p < 0.05$). We feel that the most attractive explanation for these deviations is the breeding history of the Reinhardswald population. Hungarian and Hungarian × Yugoslavian red deer were released in the first half of the 1980s. It is supposed that the autochthonous Reinhardswald population and the population of immigrants differed as to allelic frequencies at the locus concerned (Reinhardswald: high frequency of *Sod-2*^{118}, immigrants: high frequency of *Sod-2*^{100}). Thus, a deviation of the frequencies of both alleles towards an intermediate frequency and an increase of heterozygotes should have occurred in the offspring. In the subsequent years, 40% of the released deer were either culled erroneously or died in car accidents. Because of the inability to distinguish them from autochthonous deer, also the number of hybrids should have been reduced by hunting. This

artificial selection against the allele "*100*" is likely to have resulted in an increase of the autochthonous allele "*118*".

The rare allele "*100*" at the *Mpi-1* locus was found exclusively in the Reinhardswald. Also the presence of this allele may be due to the release of foreign deer described above. Alternatively it may have newly arisen by mutation.

The lack of significant allele frequency differences confirms the assumption of migration between the two Swiss red deer regions. Thus, the Walensee, the motorway V3 and the mountain ranges Churfirsten and Alvier cannot be regarded as borders preventing gene flow. However, also genetic similarity among the founder individuals of these two populations and genetic drift acting in the same direction have to be taken into account.

Relative genetic differentiation among German red deer populations (G_{ST} = 3.9%) is lower than among populations of Sweden, Norway, and Scotland (G_{ST} = 5%, Gyllensten *et al.* 1983), and among populations of Hungary, Austria, and France (G_{ST} = 7.9%, Hartl *et al.* 1990). Even lower values were obtained by Herzog (1988, F_{ST} = 1%) between the German populations Lüneburger Heide, Harz, Solling, and the northern part of Hessen.

The dendrogram (Fig. 3) demonstrates a close genetic relationship among the neighbouring populations Rothaargebirge – Dill-Bergland and Spessart – Werra--Fulda. Nevertheless, a division of the German red deer population into subpopulations must be assumed because of several motorways running through our study area, which prohibit or at least reduce migration (Fig. 1a). Also the Reinhardswald population, being in an enclosure, is reproductively isolated from the other red deer stocks. The existence of subpopulations is supported by a test for Hardy--Weinberg equilibrium. In contrast to the situation in single samples, pooling of all German red deer yielded a significant deviation ($p < 0.01$) of observed genotypic proportions from the expected ones. The excess of homozygotes observed in our sample indicates the presence of a "Wahlunds effect".

Small, isolated populations are known to be exposed to the loss of rare alleles. To preserve a certain level of genetic variability in the present red deer stocks, population sizes have to be increased at least indirectly. This can be accomplished by establishing contact among populations. Instead, the present law requires rigid separation of red deer areas. These demands should be reconsidered from a population genetic point of view – no further isolation, but more exchange among red deer populations is necessary. Therefore the establishment of a few red deer territories with several red deer districts within each of them is conceivable. Migration between those districts should be allowed.

The fundamental question arises as to what extent genetic depletion affects the survival of a population. Comprehensive investigations on correlations between genetic variability and fitness are available in domestic stock but are rare in free ranging animals. Especially as long as there is vagueness with respect to this question, all precautions have to be taken to prevent the loss of alleles in isolated populations.

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