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Genetic variability of roe deer *Capreolus capreolus* in Italy: electrophoretic survey on populations of different origin

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In order to investigate genetic variability and differentiation of roe deer *Capreolus capreolus* (Linnaeus, 1758) in Italy, four populations were examined for polymorphism at thirty-two gene loci by means of vertical polyacrylamide gel electrophoresis. Mean values of polymorphism (mean P = 15.6%, SD 4.4%) and expected average heterozygosity (mean H_e = 4.7%, SD 1.9%) were comparable to those reported previously for roe deer populations of Central Europe. Moreover, the results confirmed that roe deer is one of the genetically most variable deer species yet studied. Nei's standard genetic distances (mean D = 0.008, SD 0.004) were consistent with the values generally obtained at the level of local populations in deer species. Estimates of relative genetic differentiation showed that 11.5% of the total diversity can be assigned to genetic divergence among populations. Differences in levels of genetic variation among the Italian populations studied are discussed with respect to their respective demographic origin and historical background. The implications of our data for the management of roe deer in Italy are outlined.

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Introduction

In contrast to various other cervids, population genetic investigations in the roe deer have been started rather late and the samples studied so far covered only a small part of the large distribution area of this species. Genetic variability and differentiation have been examined in populations dwelling in and around the northern Alps and the Balkan and, with a very scattered distribution of sampling areas, in some other parts of Europe (Baccus *et al.* 1983, Hartl *et al.* 1991, 1993, Wehner *et al.* 1991). Data on roe deer populations of Southern Europe are completely lacking.

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Also the systematics of the genus *Capreolus*, especially at the subspecies level, is still not clearly understood (Honaki *et al.* 1982, Danilkin 1985, Markov *et al.* 1985). Up to now, two valid species have been recognized, the European roe deer *Capreolus capreolus* (Linnaeus, 1758), which is considered a monotypic species, and the Siberian roe deer *Capreolus pygargus* (Pallas, 1771), which has been classified into three subspecies (Sokolov and Gromov 1990).

In 1925 Festa suggested a separate subspecies status for roe deer of Central Italy (*C. c. italicus* Festa, 1925). A morphological analysis conducted on body size and hair traits of seven samples belonging to the estate of Castelporziano led the author to the conclusion that they were clearly different from the other Italian roe deer of the Eastern Alps (*C. c. transylvanicus* Matschie, 1907). However, at present all the Italian populations of roe deer are recognized as belonging to the nominate form *Capreolus capreolus capreolus*.

The distribution of roe deer in Italy had strongly declined throughout the last two centuries. Once present all over the country, it had already disappeared from almost all the peninsula by the last century (Ghigi 1911). Later on, a lot of nuclei were founded by reintroducing animals from different areas of Europe. Moreover, the autochthonous nuclei grew naturally, so that the distribution of roe deer all over Italy has increased again. Nowadays roe deer is distributed mainly in the Eastern Alps while in the Western Alps it is present at lower densities and with a patchy distribution (Fig. 1). The second large concentration of the species is found in the Northern Apennines, and in Tuscany, Central Italy. Certainly autochthonous populations are present in the headland of the Gargano, in the presidential estate of Castelporziano, near Rome, and in the Orsomarso mountains, Southern Italy.

The present study, carried out by multilocus allozyme electrophoresis, was aimed at providing data on genetic variability of the species in Italy, where no previous works are available. Furthermore, we analyzed in detail the extent of genetic variation and differentiation between four Italian populations of roe deer. Differences in levels of genetic variability found among those populations were interpreted in the light of their respective demographic origin and historical background.

Material and methods

A total of 183 roe deer belonging to two populations in Central Italy and to two populations in alpine areas (Fig. 1) were studied.

One sample came from a population living in the province of Arezzo, where roe deer is supposed to be autochthonous. This population, developed from the autochthonous nucleus of the Foreste Casentinesi in the North-Eastern Apennines, had experienced some bottlenecks during the last centuries. Isolated from the southern populations for almost one century, a few years ago it started to spread towards the north-east of Tuscany. Apart from a post-war release of four individuals from Tarvisio Forest (Lovari *et al.* 1991), there is no evidence for introductions of roe deer from other stocks.

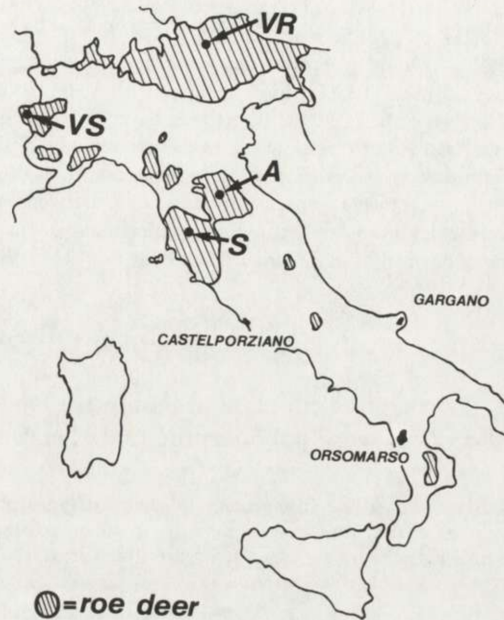

Fig. 1. Distribution area of roe deer in Italy (shaded areas) and sampling sites of the present study. A – Arezzo, S – Siena, VR – Val Rendena, VS – Val di Susa.

The sample from Siena came from a population founded both by autochthonous roe deer from the Latium Maremma and by allochthonous animals introduced from the north and the east of Europe (Mazzoni Della Stella 1991). Restockings in the south of the province had occurred since the beginning of this century. Later on, the newly founded population expanded towards the north of the province so that at present its distribution area overlaps slightly with that of the Arezzo population (Fig. 1).

The Val Rendena sample came from the Eastern Alps, where today the largest Italian population of roe deer lives. This population encountered important bottleneck events, especially during the last two wars, but it neither remained small for a longer time nor was it isolated from all other south alpine populations at any time. No introductions have been reported.

The Val di Susa sample belonged to a population that today is present at a mid-high density (approximately 30 individuals/100ha), but that originated from about 30 founders reintroduced from former Yugoslavia into a protected area of the valley in the mid-sixties (De Meneghi *et al.* 1987). From those parts of the Alps roe deer had completely disappeared for more than a hundred years.

Since their history is well documented, electrophoretic data from these last two populations were used as reference for interpreting those obtained from the Siena and the Arezzo populations.

Tissue samples (liver, heart, muscle), were collected during the hunting seasons 1989 and 1990 soon after death of the specimens and stored at -20^{0}C until electrophoresis. About 0.5g of each tissue were homogenized in 1ml of 0.01 M Tris/HCl pH 7.5, 0.001 M Na_2 – EDTA, and 0.001M ß-mercaptoethanol buffer and centrifuged at 12.000 rpm for 15 minutes. Supernatants were diluted in one volume of 40% glycerol solution and stored at -80^{0}C until used. Vertical polyacrylamide gel electrophoresis was employed under different buffer conditions to resolve 23 enzyme systems and six non-enzymatic heart proteins, encoded by 32 presumptive gene loci (Table 1). Staining recipes were adapted from Shaw and Prasad (1970), Harris and Hopkinson (1976), and Grunbaum (1981). Peptidase-B and peptidase-A were stained using respectively L-leucyl-glycyl-glicine and L-leucyl--alanine as substrates. Electromorphs were presumed to have a simple genetic basis and considered alleles. A locus was considered polymorphic if the frequency of the most common allele was less than 0.99 in any sample. Alleles were coded alphabetically according to the migration distance of the corresponding allozymes from the starting point, with the most anodal coded as "*a*".

The BIOSYS-1 program (Swofford and Selander 1989) was used to compute allele frequencies and indices of genetic variation for each population: observed (direct count) and expected (based on Hardy-Weinberg equilibrium) average heterozygosity (H_o and H_e, respectively), the proportion of polymorphic loci (P), and the mean number of alleles per locus (n). Agreement of genotypes with Hardy-Weinberg expectations was tested with χ^2 goodness-of-fit analysis (Sokal and Rohlf 1981). In order to evaluate genetic relationships among populations, the matrix of Nei's (1972) standard genetic distances and genetic identities was computed. To examine levels of population structuring and to estimate relative genetic differentiation between populations, F-statistics (Nei 1977, Wright 1978) were calculated for each polymorphic locus. Deviations of F_{ST}- and F_{IS}-values from zero were tested for statistical significance by means of a χ^2-test (Workman and Niswander 1970, Nei 1977).

Results

Twenty five out of 32 presumptive loci scored were monomorphic in all samples. Seven loci were polymorphic in at least one of the samples (Table 1). At four loci

Table 1. (a) Allele frequencies at seven polymorphic loci in four Italian roe deer populations (E.C. number and buffer system used are given in parentheses). (b) List of monomorphic loci studied (E.C. number and buffer system used are given in parentheses). (c) References for buffer systems used.

(a)

Locus	Allele	Populations			
		Siena	Arezzo	Val Rendena	Val di Susa
Me-2	*a*	0.385	0.035	0.0	0.0
(1.1.1.40, A)	*b*	0.615	0.965	1.0	1.0
6-Pgd	*a*	1.0	1.0	0.857	1.0
(1.1.1.44, D)	*b*	0.0	0.0	0.143	0.0
Hk-2	*a*	0.028	0.0	0.0	0.045
(2.7.1.1, A)	*b*	0.0	0.048	0.0	0.0
	c	0.972	0.952	1.0	0.955
Ak-1	*a*	0.699	0.862	0.536	1.0
(2.7.4.3, A, B)	*b*	0.301	0.138	0.464	0.0
Pgm	*a*	0.770	0.965	0.643	1.0
(2.7.5.1, B)	*b*	0.230	0.035	0.375	0.0
Pep-B	*a*	0.684	0.572	0.679	0.700
(3.4.11, E)	*b*	0.316	0.428	0.321	0.300
Mpi	*a*	0.043	0.154	0.321	0.136
(5.3.1.8, C)	*b*	0.957	0.846	0.679	0.864

(b) *Hb-1*, *-2* (A), *Tf* (A), *Gp-1*, *-2*, *-3* (A), *Adh-1*, *-2* (1.1.1.1, A), *Ldh-1*, *-2* (1.1.1.27, A), *Gdh* (1.1.1.47, B), *G-6-pd* (1.1.1.49, A), *Mdh-2* (1.1.1.37, B), *Xdh* (1.2.3.2, A), *Dia-1* (1.6.4.2, B), *Pox-2* (1.11.1.7, A), *Sod-1* (1.15.1.1, A), *Aat-2* (2.6.1.1, B), *Ck* (2.7.3.2, A), *Ak-2* (2.7.4.3 A, B), *Est-2* (3.1.1.1, F, G), *Pep-A* (3.4.11, E), *Ada* (3.5.4.4, A), *Fum* (4.2.1.2, A), *Pgi* (5.3.1.9, B).

(c) A – discontinuous Tris-Glycine pH 8.9 (Davis 1964), B – discontinuous Tris-Glycine pH 8.5 (Jolley and Allen 1965), C – Histidine-MES pH 6.1 (Maclellan 1982), D – Phosphate pH 7.0 (Harris and Hopkinson 1976), E – Lithium hydroxide pH 8.3 (Ferguson 1980, modified), F – Tris-Borate pH 8.9 (Studier 1973), G – HEPES-Imidazole pH 7.4 (Maclellan 1982).

(*Me-2*, *Pgm*, *Mpi*, *Ak-1*) allele frequencies were markedly different among populations and the incidence of two rare alleles (*6-Pgd*^{b}, *Hk-2*^{b}) was restricted to only one sample (Val Rendena and Arezzo, respectively). Only the allele frequencies at the *Pep-B* locus were quite similar in all populations studied.

Single locus tests for deviation of genotypes from Hardy-Weinberg expectations did not reveal significant results, except for *Mpi* ($p < 0.05$) and *Hk-2* ($p < 0.001$) in the Arezzo population. At the *Hk-2* locus the deviation from equilibrium is rather due to problems in the interpretation of electrophoretic results than to biological causes. Allozymes corresponding to allele '*b*' at the *Hk-2* locus migrated so closely to those representing the common allele '*c*' that the heterozygote '*b/c*' was difficult to be distinguished on the gels. Thus, an excess of homozygous genotypes in relation to Hardy-Weinberg expectations may be due to an underestimation of the number of heterozygotes in the sample.

Expected average heterozygosity (H_e, Table 2) ranged from 0.024 in Val di Susa to 0.067 in Val Rendena, with a mean value of 0.047 (SD 0.019). The proportion of polymorphic loci ranged from 0.094 in Val di Susa to 0.188 in Siena and Arezzo,

Table 2. Genetic variability in four Italian roe deer populations (sample sizes in parentheses). H_o (H_e) – observed (expected) average heterozygosity, P – proportion of polymorphic loci, n – mean number of alleles per locus. All indices are calculated over 32 loci.

	Populations				
	Siena (89)	Arezzo (69)	Val Rendena (14)	Val di Susa (11)	Mean/SD
H_o	0.056	0.032	0.051	0.024	0.041/0.015
H_e	0.058	0.038	0.067	0.024	0.047/0.019
P	0.188	0.188	0.156	0.094	0.156/0.044
n	1.2	1.2	1.2	1.1	

Table 3. *F*-statistics of four Italian roe deer populations for each variable locus. Significance of F_{IS}- and F_{ST}-values was tested by χ^2 analyses (* – $p < 0.05$, *** – $p < 0.001$, ns – not significant).

Locus	F_{IT}	F_{IS}	F_{ST}
Pep-B	0.155	0.146 ns	0.011 ns
Me-2	0.278	–0.003 ns	0.281 ***
Pgm	0.216	0.065 ns	0.162 ***
Mpi	0.177	0.112 ns	0.073 ***
6-Pgd	0.481	0.417 ***	0.111 ***
Hk-2	0.380	0.367 *	0.020 ns
Ak-1	0.203	0.035 ns	0.173 ***
Mean	0.214	0.112 ***	0.115 ***

Table 4. Matrix of Nei's (1972) genetic identities (above the diagonal) and standard genetic distances (below the diagonal).

Population	Siena	Arezzo	Val Rendena	Val di Susa
Siena		0.993	0.990	0.990
Arezzo	0.007		0.991	0.999
Val Rendena	0.010	0.009		0.987
Val di Susa	0.010	0.001	0.013	

with a mean value of 0.156 (SD 0.044). Except for Val di Susa (n = 1.1), the mean number of alleles was equal in all populations studied (n = 1.2).

The total genetic variance ($F_{IT} = 0.214$, Table 3) showed a positive value, which indicated a deviation from panmixia within the whole population. The latter was equally partitioned between the within- and the among-population components, the F_{IS}-value of 0.112 being virtually as high as the F_{ST}-value of 0.115. The inbreeding coefficient within subpopulations was significantly positive ($F_{IS} = 0.112$, $p < 0.001$), which suggested a trend for heterozygote deficiency. Taking into account the possible misscoring of *Hk-2* genotypes, heterozygote deficiency was mainly due to the *6-Pgd* locus ($F_{IS} = 0.417$, $p < 0.001$). Averaged over all loci, the fixation index ($F_{ST} = 0.115$) was significantly different from zero, indicating considerable relative gene divergence among populations (contingency χ^2 for allelic heterogeneity at all loci among samples = 220.2, $p < 0.0001$, df = 24). Thus, 11.5% of the total gene diversity were due to genetic diversity between populations while 88.5% ($1-F_{ST}$) of the total genetic diversity were due to the within-population component. Except for *Pep-B* and *Hk-2*, the differences in allele frequencies among populations were significant at $p < 0.001$ at all polymorphic loci.

Genetic distances (D) among populations ranged from 0.001 to 0.013 (Table 4), with a mean value of 0.008 (SD 0.004).

Discussion

Both with respect to polymorphism (mean $P = 0.156$) and expected average heterozygosity (mean $H_e = 0.047$), the protein variation observed in the present study is in accordance with the data on Central European roe deer reported in previous studies (Table 5). A remarkably higher value of polymorphism ($P = 0.283$) had been detected only by Wehner *et al.* (1991), which is most probably due to the small sample of loci investigated by these authors. Generally, the number and composition of proteins studied seems to have a considerable influence on estimates of genetic variation (cf Gorman and Renzi 1979, Lewontin 1985, Randi *et al.* 1990, Gillespie 1991).

Relative ($F_{ST} = 0.115$) and absolute (mean $D = 0.008$) genetic divergence are similar to the respective estimates reported previously for European roe deer populations (Nei's $F_{ST} = 0.110$, mean $D = 0.006$ – Hartl *et al.* 1991). Both F_{ST}-

Table 5. Genetic variability and distance estimates in European roe deer. Symbols as in Table 2, D – mean of pairwise Nei's (1972) standard or Nei's (1978) unbiased * – genetic distances.

Number of:		H_o	P	D	Reference
Loci	Populations	(H_e)			
40	13	0.056 (0.049)	0.158	0.006*	Hartl *et al.* 1990
14	5	0.043 (0.049)	0.283	0.008	Wehner *et al.* 1991
40	9	0.057 (0.059)	0.178	0.002*	Hartl *et al.* 1993
32	4	0.041 (0.047)	0.156	0.008	this study

Table 6. Genetic variation in some deer species (symbols as in Table 2).

Species	Number of:		P (%)	H (%)	Reference
	Populations	Loci			
Dama dama	9 – 22	30	0.0	0.0	Pemberton and Smith 1985
	1	15	6.6	1.8	Hartl *et al.* 1986
	1	51	2.0	0.6	Randi and Apollonio 1988
Cervus elaphus	22	34	7.7	2.2	Gyllensten et al. 1983
	6	28	11.9	2.7	Dratch and Gyllensten 1985
	17	34	11.4	3.5	Hartl *et al.* 1990
Alces alces	18	23	9.4	2.0	Ryman *et al.* 1980
	–	19	15.8	1.7	Baccus *et al.* 1983
Rangifer tarandus	4	34	13.2	2.2	Roed 1985
	5	35	16.0	4.9	Roed 1986
Odocoileus virginianus	8	20	35.8	7.4	Baccus *et al.* 1983
	4	57	31.6	6.2	Sheffield *et al.* 1985
	–	19	36.8	7.8	Smith *et al.* 1986

and D-values in the roe deer are among the highest estimates of genetic divergence as yet detected in deer at the level of local populations (cf Reuterwall 1980, Ryman *et al.* 1980, Gyllensten *et al.* 1983, Hartl *et al.* 1990). This may be associated with the comparatively high level of biochemical variation found in this species (Table 6). However, the patterns of differentiation found reflect the geographical structure of the study areas (see Hartl *et al.* 1991, 1993, Apollonio and Hartl 1993), breeding structure and dispersal (see Apollonio and Hartl 1993, Kurt *et al.* 1993), and population histories (this study), rather than the existence of geographically and genetically well separated subspecies or ecotypes. In the present study an overall deficiency of heterozygotes, reflected by positive F_{IS} and F_{IT} values, is most

probably due to a Wahlund effect (Maynard Smith 1989). In our case, considerable allelic differentiation among populations is most probably the result of genetic bottlenecks and the partially very different geographic origin of founder individuals in the respective populations.

Except for the mean number of alleles per locus, which is not a very powerful estimator of variability in our case (with one exception there are only two alleles per polymorphic locus), the four Italian roe deer populations show remarkable differences as to the extent of genetic variation (Table 2). According to the distribution of rare and common alleles among populations (Table 1), the lower P-values in Val Rendena and Val di Susa are not likely the result of smaller sample sizes (see also Hartl and Pucek, in press), but are rather explained by population histories.

The lowest values of polymorphism (P = 0.094) and average heterozygosity (H_e = 0.024) are found in the sample from Val di Susa. There the level of genetic variability most probably suffered from the permanently low population density and the geographic isolation in which the population has always remained since the time it was founded. However, if occasional bottlenecks are followed by rapid population growth and there is no restriction of genetic exchange with adjacent demes, high levels of genetic variation may be maintained. This seems to be the case in the Val Rendena population (P = 0.156, H_e = 0.067). In the Siena population, values of polymorphism and average heterozygosity (P = 0.188, H_e = 0.058) are still somewhat higher than in Val Rendena. This result may be explained by reintroductions, carried out both with autochthonous founders from the Latium Maremma and with animals originating from different areas in Europe (Mazzoni Della Stella 1991). Cross-breeding among animals from different source gene pools coud have ruled out any erosion of genetic variation brought about by the low number of founders. The gene pool of the Siena population may include also the alleles of an autochthonous stock that possibly never had fully disappeared. The intermediate level of average heterozygosity (H_e = 0.038) in the sample of Arezzo does not reveal any pronounced effects of drift, outbreeding or massive gene flow. The geographical isolation of the population could account for this result, supporting the view of a presumed autochthonous origin for the Arezzo population.

Biochemical-genetic data are useful in the management of populations living under natural conditions (Frankel and Soulé 1981, Lorenzini *et al.* 1991, Stüwe *et al.* 1991). The following suggestions for the management of roe deer in Italy emerge from our data. First of all, we propose to avoid any introductions of allochthonous individuals into the territory of Arezzo, in order to prevent the pollution of the gene pool of a most probably autochthonous population. Moreover, for any further establishment of new roe deer populations in Central Italy we recommend to use animals form Arezzo, but not from Siena. This would allow the preservation and spread of an autochthonous gene pool. For restocking operations we emphasize the necessity of using a genetically heterogeneous group of founders (checked by screening allozymes from blood or from biopsies of captured animals),

in order to provide the new population with as many alleles as possible from the parental gene pool. Allelic diversity, not high heterozygosity alone, is important for increasing the chances for long-term survival of a population (see Hartl and Pucek, in press).

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