

Allozyme variation in native red deer *Cervus elaphus* of Mesola Wood, northern Italy: implications for conservation

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Screening of 43 protein loci was used to evaluate the level of genetic variability in 42 red deer *Cervus elaphus* Linnaeus, 1758 from Mesola Wood, the only native population presently occurring in peninsular Italy. The survey revealed polymorphism at four loci. In one of them evidence was provided for a significant deficiency of heterozygotes. Average expected heterozygosity and the proportion of polymorphic loci were $H_e = 0.025$ and $P = 0.093$, respectively. Although these values are low, they are consistent with those reported for other European red deer populations. Repeated bottlenecks and slow recoveries along with prolonged selective removal of stags were indicated as affecting genetic variation as a consequence of random drift. A genetic factor may have influenced female fertility and antler conformation. Comparisons with free-ranging deer from the Alps, and with an enclosed population yielded estimates of absolute (mean Nei's 1972 $D = 0.004$, $SD = 0.002$; mean modified Rogers' $D = 0.063$, $SD = 0.013$) and relative (Wright's $F_{ST} = 0.094$) genetic distance typical for red deer populations. Given the biological value of Mesola red deer, genetic results are discussed in relation to both population history and conservation. A strategy for urgent management interventions is also proposed.

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Introduction

The "Gran Bosco della Mesola" Natural Reserve harbours the only native population of red deer *Cervus elaphus* Linnaeus, 1758 in the Italian peninsula (Castelli 1941, Mattioli 1990). All other extant populations originate either from natural recolonisation from neighbouring countries (central and eastern Alps) or from reintroductions (western Alps and Apennines). The sea and the malarial swamps that surrounded the Mesola Wood made it hardly accessible to man and saved the deer from extinction. In fact, this is one of the few European red deer populations that has managed to preserve its purity as it has never been subjected to traditional "blood-stock" improving procedures. This fact is particularly noteworthy,

since red deer has suffered more than other game species from restocking operations for sporting purposes (Beninde 1940, Whitehead 1964, Mystkowska 1966, Lowe and Gardiner 1974, Geist 1989). On the other hand, Mesola red deer have sustained several human interferences. Isolated from all other populations for the past 3–7 centuries, this nucleus has always been confined to a restricted forest (2500 ha in 1800, 1100 ha in 1900). It has been subjected to the effects of flooding, disease, hunting (until 1940), poaching, logging, competition of fallow deer and domestic animals, thus experiencing a number of demographic crashes and slow recoveries.

Mesola red deer has modest body size, a simple antler design, and a low reproductive success (Mattioli 1990, 1993a, b). Environmental restraints are obvious: scarce food resources supplied by an unproductive habitat have selected “maintenance phenotypes” (*sensu* Geist 1987), characterized by reduced stature, limited sexual dimorphism, and low recruitment. Contingent factors such as present heavy food competition by fallow deer, with consequent habitat deterioration, have further exacerbated the effects. The influence of genetic factors on the performance of this population is more controversial. Recently, a genetic component has been suggested for both the low female fertility and the antler conformation (Mattioli 1993a, b). The trophy-oriented shooting practiced throughout the years has eliminated the best stags, probably contributing to the development of an oversimplified antler structure (with bez tine and crown constantly absent over the past 40 years).

The purposes of the present study are: (1) to describe genetic polymorphism within the Mesola population; (2) to estimate the extent of genetic differentiation between Mesola and other Italian populations of red deer; (3) to evaluate the role of population history (autochthonous origin, multi-century isolation, population dynamics, selective removal) on the genetic characteristics of Mesola red deer, and (4) to develop a strategy for conservation and management.

Material and methods

Study area

Mesola Wood is located in the Po delta area, north-eastern Italy. It covers an area of 1058 ha, of which 850 ha are accessible to deer. The Elciola enclosure (97 ha) occupies the center of the Reserve. Forest habitats account for 87% of the territory, with *Quercus ilex* as the dominant tree species. This sub-mediterranean coastal wood originated on sandy dunes in late Middle Ages during a warm climate period. Managed for centuries as a coppice, it is now slowly being converted into a high forest. Besides red deer, Mesola Wood shelters an introduced population of fallow deer, the size of which varied from 350 to 1000 individuals over the past 15 years (with an estimated biomass of 16–45 tons).

Demography

Seasonal surveys from 1982 onward have permitted a continuous monitoring of the population dynamics and of the reproductive performance of Mesola red deer, giving virtually exact numbers for the Elciola subpopulation, and reliable estimates for the other one. Population size figures concerning

the period previous to 1982 are taken from Castelli (1941), Perco (1984), and Mattioli (1990). The male segment of the effective population size (N_e) was assessed assuming that the actual breeders were only high rank adult stags (observed harem holders, 7–8 pointers, large-sized mature individuals). Opportunistic matings were excluded. The female segment was calculated considering hinds sighted with a calf at foot in summer. The yearly changes in population size are expressed as annual finite rate of increase $\lambda = e^r$ (Caughley 1977).

Genetic analysis

A total of 109 red deer from four sampling sites (Fig. 1) were analysed for electrophoretic variation. Samples of blood and skeletal muscle were collected by jugular puncture and biopsies, respectively, during 1994 through 1996 from 42 live red deer of Mesola Wood. Animals were captured by use of a dart gun and immobilizing drugs. Whole blood, serum or tissues (liver, heart) were obtained from caught or dead animals from Tarvisio ($n = 32$) and Stelvio ($n = 13$), eastern and central Alps, respectively. A fourth sample comprised red deer from an enclosure near Lucca ($n = 22$), central Italy. The latter is a mixed population, founded by individuals from different parts of the Alps, and by animals of unknown origin coming from zoos and private parks.

Blood cells and plasma were separated by centrifugation at 3000 rpm. Some aliquots of plasma were diluted 1:10 with distilled water before storing at -80°C . Red blood cells were frozen and thawed twice, then an equal volume of deionized water was added. About 0.5 g of heart, liver and muscle were

homogenized in 1 ml of 0.01 M Tris/HCl pH 7.5, 0.001 M Na₂EDTA, 0.001 β -mercaptoethanol buffer, and centrifuged at 15000 rpm at $+4^\circ\text{C}$. Supernatants were diluted in an equal volume of 40% glycerol solution, and stored at -80°C until analysis. Electrophoresis was performed at $+4^\circ\text{C}$ on vertical and horizontal polyacrylamide gels using different electrophoretic conditions (Lorenzini *et al.* 1993) and staining recipes (Harris and Hopkinson 1976, Murphy *et al.* 1990). Hemoglobin was separated by isoelectric focusing in 0.5 mm acrylamide gels, using a pH 5.5–8.5 gradient (Pharmacia). General proteins were stained with Coomassie Brilliant Blue R-250. The following 43 putative gene loci were consistently resolved in at least one population (E.C. numbers and tissues are given in parentheses: H – heart, L – liver, M – muscle, Rbc – red blood cells, P/S – plasma or serum): *Aat-1*, -2 (2.6.1.1, H, M), *Acp-1*, -2 (3.1.3.2, Rbc), *Ada-1*, -2 (3.5.4.4, H, M), *Alb* (P/S), *Aldo* (4.1.2.13, H, M), *Ak-1*, -2 (2.7.4.3, H, M), *Ca-1* (4.2.1.1, H, M), *Ca-2* (4.2.1.1., Rbc), *Ck-1*, -2 (2.7.3.2, H, M), *Dia-1*, -2 (1.6.2.2, Rbc), *Est-1*, -2 (3.1.1.1, P/S), *Est-D* (3.1.1.1, Rbc), *Fh* (4.2.1.2, H, M), *General protein-1*, -2 (H), *Gpi* (5.3.1.9, H, M), *Hb-1*, -2 (Rbc), *Hk* (2.7.1.1, H,

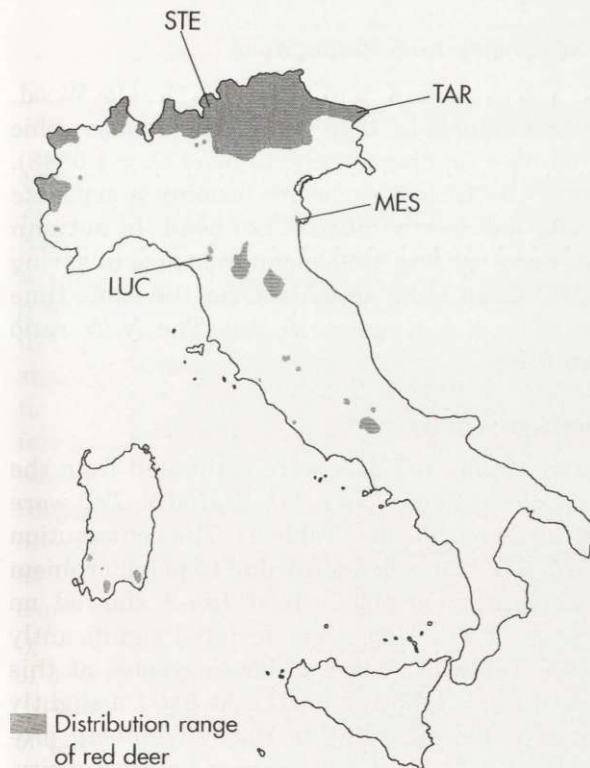


Fig. 1. Sampling sites of red deer in Italy. MES – Mesola, STE – Stelvio, TAR – Tarvisio, LUC – Lucca.

M), *Idh-1*, -2 (1.1.1.42, H, M), *Ldh-1*, -2 (1.1.1.27, H, M), *Mod-1*, -2 (1.1.1.40, H, M), *Mor-1*, -2 (1.1.1.37, H, M), *Mpi* (5.3.1.8, Rbc, H, M), *Pep-A*, -*B* (3.4.11, H, M), *Pgd* (1.1.1.44, Rbc, L), *Pgm* (2.7.5.1, H, M), *Sod-1*, -2 (1.15.1.1, Rbc), *Trf* (P/S), *Xdh* (1.3.2.1, H, M).

Banding patterns of isozyme systems were compared on the same gel with those of red deer from central Europe analysed by Hartl *et al.* (1990). Allele frequencies were derived from allozyme genotypes on the gels. Populations were tested for deviation of genotype frequencies from Hardy-Weinberg expectations by a χ^2 -test (Sokal and Rohlf 1994) with Levene (1949) correction for small sample size ($n < 30$). Observed (H_o) and expected (H_e) average heterozygosity, and the proportion of polymorphic loci (P , 99% criterion) were calculated for each population. The significance of differences among allelic frequencies in the four populations was determined through contingency table analyses and the χ^2 statistics. Heterogeneity among populations at each variable locus was further estimated from the F_{ST} index (Nei 1977, Wright 1978), and checked for statistical significance according to Workman and Niswander (1970). Values of Nei's standard (1972) and modified Rogers' (Wright 1978) genetic distance were calculated. Unweighted pair group arithmetic average (UPGMA) method (Sneath and Sokal 1973), Wagner (Farris 1972), and Fitch-Margoliash (1967) procedures were used to construct rooted and unrooted trees on the basis of matrices of genetic distances. Computations were performed using the programs BIOSYS-1, Release 1.7 (Swofford and Selander 1989), and PHYLIP (Felsenstein 1989).

Results

Population history of red deer from Mesola Wood

In the 1930's 250–300 red deer were estimated to roam in Mesola Wood. Population size dropped to about 10 individuals in 1945–1947. The demographic recovery was so slow that in 1970 red deer numbered only 40 head ($\lambda = 1.0548$). In 1972, 12 animals managed to enter the Elciola enclosure forming a separate subpopulation. In autumn 1980 Mesola red deer numbered 120 head. In autumn 1982 they dropped to 90 ($\lambda = 0.9086$). From 1982 to 1998 population size in spring decreased annually by 2.6% ($\lambda = 0.974$) down to 59 animals. Over the same time span N_e averaged 15.2 individuals (SD = 2.9, range = 10–20). The N_e/N ratio ranged between 0.17 and 0.26 (mean 0.26).

Genetic variability

Parameters of genetic variability in Mesola red deer were estimated from the analysis of 43 presumptive gene loci. Four of them (*Est-1*, *Idh-2*, *Mod-2*, *Trf*) were polymorphic, with two alleles occurring at each locus (Table 1). The contribution to average observed heterozygosity ($H_o = 0.023$) was mainly due to polymorphism at *Mod-2* and *Est-1*. *Trf* was only slightly variable, whilst *Idh-2* showed no heterozygotes and genotypic proportions at this locus thus deviated significantly from Hardy-Weinberg proportions (see Table 1). A lack of heterozygotes at this locus was also indicated by the value of $F_{IS} = 1.00$ ($p < 0.001$). At *Est-1* a slightly larger number of homozygotes than expected according to Hardy-Weinberg proportions was found ($p < 0.08$, $\chi^2 = 3.0$, $df = 1$). Values of average heterozygosity, the proportion of polymorphic loci, and the mean number of alleles per locus are also given in Table 1.

Table 1. Allele frequencies, observed (expected) single locus heterozygosities (h) at four polymorphic loci, and indices of genetic variability (calculated over 43 loci) in 42 red deer from Mesola Wood. H_o (H_e) – observed (expected) average heterozygosity, P – proportion of polymorphic loci, n – mean number of alleles per locus. ns – not significant, ** – $p < 0.001$. ^a – all values with 1 df.

Locus	Allele	Frequency	h	Chi-square ^a
<i>Mod-2</i>	<i>a</i>	0.682	0.545 (0.434)	1.5 ns
	<i>b</i>	0.318		
<i>Idh-2</i>	<i>a</i>	0.955	0.000 (0.087)	22.0 **
	<i>b</i>	0.045		
<i>Est-1</i>	<i>a</i>	0.308	0.308 (0.426)	3.0 ns
	<i>b</i>	0.692		
<i>Trf</i>	<i>a</i>	0.936	0.128 (0.120)	0.2 ns
	<i>b</i>	0.064		
H_o (H_e)			0.023 (0.025)	
P			0.093	
n			1.1	

Genetic structure and differentiation of populations

Because of the availability of serum or whole blood, but not of tissues for many samples, only 19 protein loci could be consistently scored in all populations (Table 2). Polymorphism was detected at four loci (*Est-1*, *Trf*, *Mpi*, *Ca-2*) in at least one population. *Sod-2* was variable in Stelvio (expected single locus heterozygosity, $h_e = 0.335$, $p > 0.01$ for Hardy-Weinberg equilibrium) and Tarvisio ($h_e = 0.069$, $p > 0.001$), and monomorphic in Mesola. However, being not consistently scorable in Lucca, this locus was not included in the computations. Allele frequencies differed significantly between populations, and F_{ST} values at single loci ranged from 0.055 to 0.150, with $p < 0.01$ for all loci. Mean F_{ST} revealed significant genetic differentiation also across loci among populations ($F_{ST} = 0.094$, $p < 0.001$). This value, which was mainly due to differences at *Trf* and *Ca-2* ($F_{ST} = 0.130$ and 0.150, respectively), indicates that at least 9% of the total variability is attributable to divergence between populations. Private alleles (*sensu* Slatkin 1985) were found at *Mpi* in Stelvio and at *Ca-2* in Lucca, with relatively low frequencies. Departure from equilibrium was revealed at the *Est-1* locus in the population of Stelvio ($\chi^2 = 7.0$, $df = 1$, $p < 0.01$), and at *Ca-2* in Lucca ($\chi^2 = 3.7$, $df = 1$, $p < 0.05$). Estimates of overall genetic variability in all populations are given in Table 2.

Comparisons between samples from Mesola and the other red deer, regardless of population subdivision (computations could be made on the basis of 37 loci), yielded estimates of observed (expected) heterozygosity of 0.012 (0.017) and 0.033 (0.035), respectively, for Mesola and the pooled populations. P was 0.081 in Mesola versus 0.189 in the combined sample. A mean F_{ST} of 0.090 (significantly different

Table 2. Allele frequencies at four polymorphic loci, and parameters of genetic variability (calculated over 19 loci, *Sod-2* excluded) in four red deer populations from Italy. H_o (H_e) – observed (expected) average heterozygosity, P – proportion of polymorphic loci, F_{ST} – fixation index (Wright 1978), * – $p < 0.01$, ** – $p < 0.001$. ^a – monomorphic loci: *Sod-1*, *Hb-1*, -2, *Acp-1*, -2, *Alb*, *General protein-1*, -2, *Pgm*, *Pgi*, *Est-2*, *Est-D*, *Pgd*, *Dia-1*, -2.

Locus ^a	Allele	Populations				F_{ST}
		Mesola ($n = 42$)	Stelvio ($n = 13$)	Tarvisio ($n = 32$)	Lucca ($n = 22$)	
<i>Est-1</i>	<i>a</i>	0.308	0.125	0.065	0.237	0.059*
	<i>b</i>	0.692	0.875	0.935	0.763	
<i>Trf</i>	<i>a</i>	0.936	0.708	0.935	1.0	0.130**
	<i>b</i>	0.064	0.292	0.065	0.0	
<i>Mpi</i>	<i>a</i>	0.0	0.071	0.0	0.0	0.055*
	<i>b</i>	1.0	0.929	1.0	1.0	
<i>Ca-2</i>	<i>a</i>	1.0	1.0	1.0	0.810	0.150**
	<i>b</i>	0.0	0.0	0.0	0.190	
<i>Sod-2</i>	<i>a</i>	0.0	0.214	0.036	not scored	–
	<i>b</i>	1.0	0.786	0.964		
H_o		0.023	0.034	0.014	0.024	
H_e		0.029	0.042	0.013	0.036	
P		0.105	0.158	0.105	0.105	

from zero at $p < 0.001$) was quite the same as the value obtained when the four populations were considered separately ($F_{ST} = 0.094$). The same holds for genetic distances, which showed quite similar values when calculated considering either two (Nei's $D = 0.005$, modified Rogers' $D = 0.072$) or four populations (mean Nei's $D = 0.004$, SD = 0.002, mean modified Rogers' $D = 0.063$, SD = 0.013; Table 3). A Wagner tree obtained by rooting at midpoint of the longest path is displayed in Fig. 2. UPGMA and Fitch-Margoliash dendrograms based on Nei's and modified Rogers' distances (not shown) revealed the same topology as the Wagner network in Fig. 2.

Table 3. Nei's (1972) standard genetic distance, below diagonal, and modified Rogers' (Wright 1978) distance, above diagonal, between four Italian red deer populations.

	1. Mesola	2. Stelvio	3. Tarvisio	4. Lucca
1.	–	0.069	0.056	0.049
2.	0.005	–	0.056	0.086
3.	0.003	0.003	–	0.061
4.	0.002	0.008	0.004	–

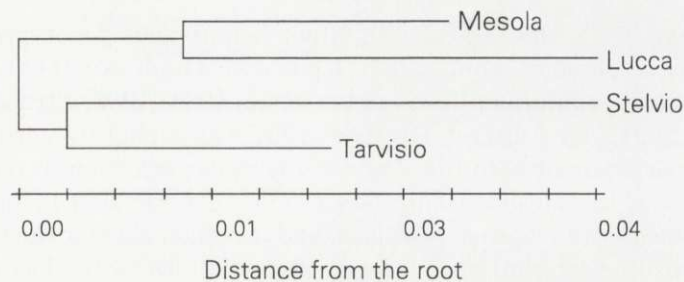


Fig. 2. Wagner tree (rooted at midpoint) based on the matrix of modified Rogers' genetic distances, displaying genetic relationships between the four red deer populations studied. Cophenetic correlation coefficient = 0.980.

Discussion

The level of genetic variability in red deer from Mesola Wood is among the lowest as yet found in *Cervus elaphus* populations analysed for allozyme variation in Europe (Baccus *et al.* 1983, Dratch 1983, Dratch and Gyllensten 1983, Gyllensten *et al.* 1983, Hartl *et al.* 1990, 1993, Ströhlein *et al.* 1993, 1994). Of four polymorphic loci, *Est-1* and *Mod-2* contributed most to average heterozygosity. In contrast, *Trf* and *Idh-2* proved to be only slightly polymorphic, although they were reported as highly variable in almost all the populations studied so far (Gyllensten 1980, Gyllensten *et al.* 1983, Pemberton *et al.* 1988, Hartl *et al.* 1990, 1993, Herzog *et al.* 1991, Stratil *et al.* 1990, Strandgaard and Simonsen 1993, Gehle and Herzog 1994, Schreiber *et al.* 1994). The low heterozygosity observed in this population for regularly highly polymorphic loci in red deer and the lack of rare alleles suggest that genetic isolation, along with repeated bottleneck events during the last centuries, have influenced both the mean level of genetic variability and the allelic diversity (see Soulé and Wilcox 1980, Frankel and Soulé 1987, Soulé 1987, for reviews).

In red deer the presence of certain alleles at the loci *Idh-2* and *Trf* turned out to be significantly associated with some morphological characters and fitness components. Individuals homozygous for the allele *Idh-2^b* showed a higher number of antler tines in males, and a larger hind foot length in females (Hartl *et al.* 1991, 1995a). Heterozygotes for *Idh-2^{ab}* were associated with a better survival of calves, in particular of female ones (Pemberton *et al.* 1988). It was suggested that some form of selection (acting on the enzyme locus itself or on genes closely linked to it) might be responsible for the maintenance of polymorphism at this locus even in small-sized populations (Pemberton *et al.* 1988, Hartl *et al.* 1991, 1995a, b). Both the poor quality of antlers and the low reproductive performance of the Mesola population may be related to the very low frequency of the *b* allele at *Idh-2* (as representative of loci associated with phenotypic traits). The same association with juvenile survival as that for *Idh-2* holds for the locus *Trf*: *Trf^{ab}* calves were

found to survive better than those with either homozygous genotypes (Pemberton *et al.* 1988). *Trf* occurred as a multiallelic locus with a high extent of heterozygosity in many red deer populations (Gyllensten *et al.* 1980, 1983, Stratil *et al.* 1990, Herzog *et al.* 1991). By contrast, in Mesola *Trf* was a slightly variable bi-allelic locus. If polymorphism at both *Idh-2* and *Trf* is under selection in red deer, it can be argued that in the Mesola population random genetic drift has countervailed any possible selective advantage of alleles, and is responsible for the trend towards fixation. Extensive variation in red deer was found also at the loci *Mod-2* (Hartl *et al.* 1991) and *Est-1* (Pemberton *et al.* 1988), but no associations with phenotypic traits could be found or tested. In Mesola red deer, frequencies of variant alleles are quite high as those found in populations from Rhum, UK (*Est-1*: Pemberton *et al.* 1988), from Austria, France, and Hungary (*Mod-2*: Hartl *et al.* 1990). These results suggest that the morphological and fitness traits described for Mesola red deer are influenced also by a genetic component which, in turn, is influenced by genetic drift resulting from bottlenecks, slow recovery rates, and variation in population size and composition. In particular, a founder effect might be involved in the simple antler architecture (Mattioli 1993b), as suggested for other deer populations (McCullough 1982, Feldhamer *et al.* 1985, Kaji *et al.* 1988). Consistent changes in the genetic structure of populations due to long-lasting hunting are also well documented (Ryman *et al.* 1981, Scribner *et al.* 1985, Hartl *et al.* 1995a).

Taking into account some possible bias due to the reduced set of loci scored in all samples, the mean level of genetic variability in the Mesola population is quite similar to that of red deer from both the Alps and Lucca. Among the four populations examined the one from Stelvio showed the highest level of genetic variation, as could be expected from its demographic history. This population had established by natural dispersal of animals from the Swiss Alps. Isolation was prevented by high rates of gene flow, overcoming the genetic consequences of founder effect and random drift. The lowest value of heterozygosity was found in the population of Tarvisio, which probably suffered from a founder effect following reintroduction in the adjacent Austrian Alps. For red deer from Lucca, which lived in an enclosure, a low heterozygosity and a decrease of allelic diversity was detected. On the other hand, a polymorphism at *Ca-2*, never found in previous studies on red deer, suggests the presence of foreign alleles in the founder stock. The somewhat closer relationship between the Lucca and the Mesola populations displayed by the Wagner tree (see also Table 3) is rather due to a common effect of drift than to a true genetic resemblance.

The values of heterozygosity obtained so far for red deer from both Italy (Mesola included) and the rest of Europe are relatively low compared to other deer species (see Hartl *et al.* 1990, Dratch and Pemberton 1992, Ströhlein *et al.* 1994 for reviews). Taking into account that red deer is one of the most intensively managed game species in Europe, this is likely to be explained by habitat fragmentation, hunting, and reintroductions, which confined red deer in small and isolated populations, often affected by erosion of genetic variability.

Relative genetic differentiation and absolute genetic distances obtained in this study were small between populations, suggesting that red deer from Mesola Wood clearly belong to the subspecies *C. e. elaphus*. Distances are consistent with the values found for other free-ranging and enclosed populations of red deer in Europe (Gyllensten *et al.* 1983, Hartl *et al.* 1990, 1991). However, in red deer the subspecies status, as suggested by morphological criteria (Groves and Grubb 1987), is difficult to define by biochemical (Strandgaard and Simonsen 1993) and molecular (Hartl *et al.* 1995b) methods. Apart from the subspecies status, the importance of preserving locally adapted populations, like Mesola red deer, is becoming more commonly accepted. Conservation measures should be adopted urgently to favour the growth of this Italian native population, thus minimizing the risk of further loss of genetic diversity and preventing the population from ultimate extinction (Gilpin and Soulé 1986). An increase of inbreeding across generations due to a low effective population size (N_e) is estimated by $F = 1/2N_e$, where F is defined as the rate of inbreeding per generation. In our case with mean $N_e = 15.2$, we obtain $F = 0.033$. This value is about three times the threshold of 1% at which natural selection can offset the tendency to the fixation of deleterious genes (Franklin 1980, Soulé 1980). Wright's (1969) basic formula defines the rate of loss of genetic variation in an ideal population as $H_t = H_o[1-F]^t$, where H_o is the initial heterozygosity and t is time in terms of generations. Considering $H_o = 0.023$ in the Mesola population, then H for $t = 1$ is 0.022, suggesting that 4.3% heterozygosity ($\Delta H = 0.001$) are lost per generation. If we assume that one generation lasts 8 years, less than 70% of the genetic variation still remains after 10 generations, a time span corresponding to 80 years. Owing to the influence of unpredictable factors on N_e across years (ie variance in offspring production or in male reproductive success), the loss of heterozygosity as a function of N_e could be even greater than predicted by the inbreeding formulas (Fitzsimmons *et al.* 1995).

In order to allow an increase of population size in Mesola red deer, it is of utmost importance to eliminate the most detrimental factors to its recovery as soon as possible. Fallow deer should be removed by capture and culling, a greater forage availability should be created by means of supplementary feeding during the winter, improvement of forest habitat (increasing structural and specific heterogeneity, opening clearings), and improvement of pastures (reseeding, mowing, installing temporary exclosures). New semi-natural nuclei should be established, preferentially in similar Italian areas (forest tracts in lowlands, coastal woods, submediterranean and mediterranean environments). These new populations should be managed with great care from a genetic point of view, choosing the founders, and ensuring a gene flow by means of translocations to counter genetic drift. The creation of alternative stocks becomes indispensable if we consider the sanitary risks the present unique population is facing at the moment.

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