Population Viability Analysis and Genetic Diversity of the Endangered Red Deer Cervus elaphus Population from Mesola, Italy

Authors: Zachos, Frank E., Hajji, Ghaiet M., Hmwe, San San, Hartl, Günther B., Lorenzini, Rita, et al.

Source: Wildlife Biology, 15(2) : 175-186

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/07-075

Wildl. Biol. 15: 175-186 (2009)
DOI: 10.2981/07-075

www.wildlifebiology.com

Original article

Population viability analysis and genetic diversity of the endangered red deer *Cervus elaphus* population from Mesola, Italy

Frank E. Zachos, Ghaiet M. Hajji, San San Hmwe, Günther B. Hartl, Rita Lorenzini & Stefano Mattioli

The Mesola red deer *Cervus elaphus* in the Po delta are the only native red deer population on the Italian mainland and have been the focus of conservationists and wildlife biologists for some time. In our study, we present a genetic analysis of 25 Mesola red deer on the basis of 20 polymorphic microsatellite loci, aiming at estimating the population's genetic diversity and at providing information for a future genetic screening. In addition, we carried out a population viability analysis (PVA) with demographic and life-history data available from a long-term population survey, simulating different management scenarios. Genetic diversity was very low compared to the rest of Europe (observed and expected heterozygosity 0.50 and 0.61, respectively), and an overall excess of homozygosity was indicative of inbreeding. Calculations of the probability of identity and genotype mismatch frequencies suggested that between five and seven highly informative loci were sufficient to resolve individuals with reasonable certainty. The PVA yielded a generally poor outlook, but at the same time, it showed that management measures already taken significantly increased population viability. A sensitivity analysis revealed that inbreeding depression and possible catastrophes had a huge impact on the population's prospects. However, the establishment of two subpopulations and successful attempts at reducing the consequences of catastrophic events were able to significantly mitigate the harmful effects of both inbreeding and environmental stochasticity. These results, in particular the splitting of the population, may be of general interest to conservationists dealing with unique threatened populations.

Key words: Cervus elaphus, Mesola, microsatellites, population viability analysis, red deer

Frank E. Zachos & Günther B. Hartl, Zoological Institute, Christian-Albrechts-University, Olshausenstrasse 40, D-24118 Kiel, Germany - e-mail addresses: fzachos@zoologie.uni-kiel.de (Frank E. Zachos); ghartl@zoologie.uni-kiel.de (Günther B. Hartl)
Ghaiet M. Hajji, U.R. de Biologie Animale et Systématique Évolutive, Département de Biologie, El Manar, Tunisia - e-mail: gayahajji@hotmail.com
San San Hmwe, (92) Khyne Shwe War Street (Baho Road), Three Quarter Kamayut Township Yangon University Post Office Yangon, Myanmar - e-mail: sansan.hmwe@gmail.com
Rita Lorenzini, the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana Unità Specialistica di Medicina Forense Veterinaria Terrestre e Marina Sezione di Rieti, Via Tancia 21, I-02100 Rieti, Italy - e-mail: rita.lorenzini@izslt.it
Stefano Mattioli, Research Unit of Behavioural Ecology, Ethology and Wildlife Management, Department of Environmental Sciences, Via Pendola 62, I-53100 Siena, Italy - e-mail: stef.mattioli@libero.it

Corresponding author: Frank E. Zachos

Received 6 October 2007, accepted 17 October 2008

Associate Editor: Paul Wilson

Conservation and management of small isolated populations commonly face problems like inbreeding, genetic drift and high susceptibility to catastrophes, diseases and environmental stochasticity in general. Populations of polygynous large mammals with their comparatively low effective population sizes and long generation times are particularly vulnerable. One such population is the red deer *Cervus elaphus* population from the 'Gran Bosco della Mesola' natural reserve, located in the Po delta

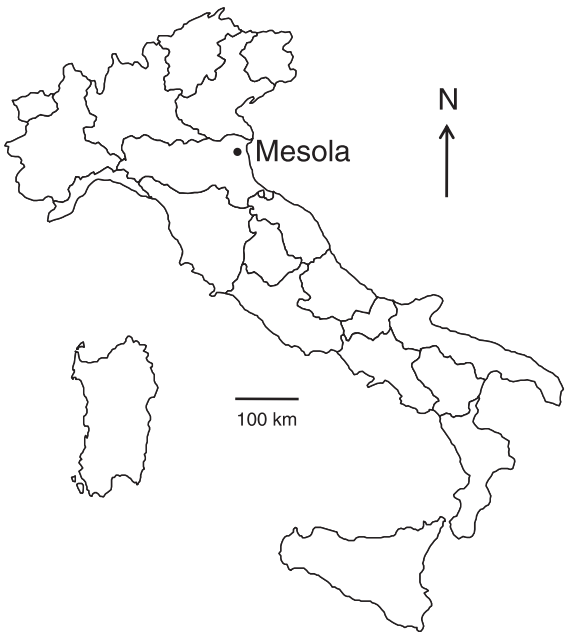

Figure 1. Geographical location of Mesola Wood.

area in northern Italy (Fig. 1), which has been isolated from other red deer populations for centuries (Mattioli 1990, Mattioli et al. 2003). It is the only remaining native red deer population of the Italian peninsula, which was one of the main glacial refugia for many temperate species, and which has been shown to harbour genetically unique populations of another cervid species, the roe deer *Capreolus capreolus* (Lorenzini et al. 2002). Apart from the Mesola red deer, all other extant red deer populations of the Italian mainland were subsequently founded by immigrating animals from neighbouring countries (eastern Alps) or through human reintroduction (western Alps and Apennines) (Mattioli 1990). Therefore, the Mesola population has been the focus of conservationists and wildlife managers for some time.

In 1922, the Mesola population numbered about 160 animals and increased to 250-300 individuals in the 1930s, but after the Second World War, only 10 red deer survived (Mattioli 1990). In 1970, there were again about 40 animals, but after an increase to 120 individuals in 1980, numbers dropped to 54 in 1992, and in 1999, population size was 67 (Lorenzini et al. 1998, Mattioli et al. 2003). The latest estimate was 120 animals in 2006 (S. Mattioli, unpubl. data).

The Mesola red deer have been monitored since 1982, and a large data set on population dynamics, reproductive performance and morphological characteristics has been collected (Mattioli 1990, Mattioli et al. 2003). Mesola red deer represent 'maintenance phenotypes' (Geist 1987), which is typical of populations living in resource-restricted habitats, with modest body size, simplified antler structure, low fecundity and low calf survival. This maintenance phenotype has probably been a characteristic feature of these deer for centuries as a consequence of a habitat of sclerophyllous wood on sandy soil with scarce herb and shrub layers (Mattioli et al. 2003). Later, their poor performance was further exacerbated by hunting and competition with introduced fallow deer *Dama dama* (up to 1,000 animals in the Mesola wood in the 1980s, and now it is between 400-450). Detailed analyses showed that the Mesola red deer had 35-40 calves per 100 hinds per year on average (as opposed to a usual figure of 50-70 calves), and showed slow body growth and delayed antler development with some antlerless yearlings and subadults and with adult antlers lacking the bez tine and the crown (Mattioli 1990,1993, Mattioli et al. 2003, S. Mattioli, unpubl. data). The Mesola red deer antlers are even more simplified than those of red deer from other poor habitats such as the Sardinian maquis scrub or the Scottish Highlands (Mattioli 1993). Interestingly, management measures taken from 1994 onwards (supplementary feeding during winter, recurrent pasture mowing, reseeding of sample areas, and the removal of several hundred fallow deer) resulted in an increase in mean body weight and calving rate, and a decrease in mortality (Mattioli et al. 2003). Moreover, the quality of antlers was also improved, the number of antlerless yearlings decreased, and for the first time in 40 years, stags with a bez tine and/or a rudimentary crown occurred (Mattioli et al. 2003). This suggests that the poor environmental conditions at least partly account for the comparatively poor physical and reproductive performance of the Mesola red deer. However, given the demographic history of repeated bottlenecks and a very low long-term effective population size, inbreeding is likely to further reduce the animals' fitness (for examples of inbreeding depression in red deer, see Slate et al. 2000 and Zachos et al. 2007). A thorough population genetic analysis of the Mesola red deer has been explicitly demanded (Mattioli 1990), and some preliminary studies have already been carried out. A study based on allozymes (Lorenzini et al. 1998) found genetic variability of the Mesola population to be low, but still within the range reported for other European red deer populations, whereas

an analysis of mitochondrial restriction fragment length polymorphisms (Lorenzini et al. 2005) yielded no variation for the Mesola population. The most comprehensive study to date on the topic (Hmwe et al. 2006) confirmed the mitochondrial monomorphism with sequences of the control region and also found the lowest heterozygosity values of microsatellite loci compared to the rest of Europe.

A report written for the Italian Ministry of Agriculture and Forestry (Fico 1998) suggested a genetic screening of the animals along with ecological research in the future. In our study, we carried out a population and conservation genetic analysis of the Mesola red deer based on 20 polymorphic microsatellite loci, nearly twice as many as analysed in the previous study by Hmwe et al. (2006). This difference in locus number is important, because it has been shown that with the usual number of loci studied (around 10), calculations of population genetic parameters often tend to be unreliable (Slate & Pemberton 2002, Koskinen et al. 2004). Our genetic study aims at providing 1) a genetic underpinning for the ecological research being carried out on the Mesola red deer, and 2) a contribution to the scientific basis for the future conservation and management of this unique population.

Further, we carried out a population viability analysis using available demographic, biological and genetic data with the particular aim of estimating the population's relative susceptibility to inbreeding depression, carrying capacity and catastrophes ('sensitivity testing'), and to assess the relative effect of management measures (those already taken and others that have been suggested). In particular, we used our data set to infer the consequences of splitting the population into two subpopulations to reduce its susceptibility to environmental stochasticity. This approach is of general interest to conservationists dealing with small populations. Our case study may thus yield evidence if the high financial costs and logistic efforts usually involved in dividing a population (in particular in the case of large mammals) are justified by the improved prospects of the endangered population.

Material and methods

Sampling and genotypic data

Blood samples from 25 live-caught Mesola red deer were taken by jugular puncture between 1995 and 1998 (total population size at the time was about 60). Genotypic data from 12 polymorphic microsatellite loci analysed in a previous study (Hmwe et al. 2006), and new data from another eight loci were combined into a comprehensive data set. Microsatellites are short repetitive stretches of DNA whose alleles differ in repeat numbers and hence in length. Their high mutation rates make them popular markers for population and conservation genetic studies at the intraspecific level (e.g. Kuehn et al. 2003, Estonba et al. 2006, Redeker et al. 2006, Zachos et al. 2006). The twenty loci studied were: OarCP26, OarFCB304, OarVH110, MAF35, MAF109, INRA11, INRA121, BM203, BM757, BM888, BM4208, BM4513, BMC1009, NVHRT16, NVHRT21, RT1, RT7, BL42, IDVGA55 and TGLA53 (for more information on these loci and PCR conditions, see Bonnet et al. 2002, Zachos et al. 2003 and Hmwe et al. 2006).

Statistical analysis of the genetic data

The 20 loci were tested for pairwise linkage disequilibrium with the GENEPOP software (Raymond & Rousset 1995). Since stutter bands, large allele dropout and null alleles are known to distort allele frequencies and heterozygosity calculations (Pemberton et al. 1995, Wattier et al. 1998, Ewen et al. 2000), we tested our data set for these potential error sources with the programme MICRO-CHECKER (van Oosterhout et al. 2004). The presence of null alleles is assessed on the basis of the proportion of homozygotes. An excess of homozygotes, however, can also be caused by other factors such as inbreeding (which is particularly likely in small and bottlenecked populations like Mesola). Observed (H_O) and expected (H_E) heterozygosity as well as deviations from Hardy-Weinberg equilibrium (HWE) were calculated with Arlequin (Schneider et al. 2000). GENEPOP was used to test the allele data for an overall multi-locus heterozygote deficit. The population's inbreeding coefficient (F_{IS}) was computed with FSTAT (Goudet 1995). Allelic diversity was calculated as the mean number of alleles per locus.

To test our sample animals for potential parent-offspring relationships (which might bias estimations of genetic diversity and inbreeding), we used the 'genetic determination of kinship' function in the GIMLET software (Valière 2002). An individual is regarded as a possible parent when at least one allele per locus is identical to one found in another individual. There is also an incompatibility function where one can determine a maximum

number of allowed mismatches between parent and offspring (to take into consideration potential misidentification of alleles). GIMLET was also used to calculate the probability of identity (P_{ID}), i.e. the probability that two individuals drawn randomly from the population will show identical multi-locus genotypes. This parameter reflects the extent of genetic variability in the population and provides an estimate of the minimum number of loci needed to discriminate single individuals, which is important in genetic monitoring to not unnecessarily increase the high laboratory costs. P_{ID} was calculated using the standard equation (Paetkau & Strobeck 1994) and the equation for P_{ID} among siblings (Evett & Weir 1998) which has been suggested as a conservative upper boundary for individual identification, because normal theoretical P_{ID} values have been shown to underestimate the true probability of identity (Waits et al. 2001):

$$P_{IDstand} = \Sigma P_i^4 + \Sigma\Sigma(2p_i p_j)^2 \text{ and}$$

$$P_{IDsib} = 0.25 + 0.5\Sigma p_i^2 + 0.5(\Sigma p_i^2)^2 - 0.25\Sigma p_i^4$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles and $i \neq j$. P_{ID} values were calculated for each locus and then multiplied across loci to obtain the overall P_{ID}.

The proportion of so-called two-mismatch-pairs (2MM-pairs), i.e. pairs of genotypes that match at all but two loci, was also calculated as a predictor of power to resolve different individuals (Paetkau 2003). A 2MM proportion of 0.005 has been suggested as the threshold for acceptable resolution power when sample sizes are small, i.e. $N < 100$ (Paetkau 2003).

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

Population viability analysis

Contrary to repeated criticism, population viability analysis (PVA) has been shown to yield highly accurate predictions, and its use has been explicitly recommended to assist in managing endangered species or populations (Brook et al. 2000). We carried out a PVA with the VORTEX software (Lacy et al. 2005) using the available data on the Mesola population. This software has been shown to yield especially good results in a recent comparative study of PVA programmes (Brook et al. 2000). In particular, we conducted a sensitivity analysis to infer which parameters are most important to the population's viability. By using a range of possible values for uncertain parameters, one can determine what effects these uncertainties are likely to have on the PVA results. This approach has been praised as particularly fruitful in conservation and management (Frankham et al. 2002, Allendorf & Luikart 2007), and has been used to develop and evaluate recovery programs for threatened species or populations (e.g. Wisdom & Mills 1997, Hosack et al. 2002). We specifically tested the relative effects of inbreeding depression, catastrophes and splitting the population into two subpopulations with a higher total carrying capacity. Inbreeding depression was chosen because it is known to occur in wild red deer populations (Slate et al. 2000, Zachos et al. 2007), and genetic consequences of the long-term low effective population size and the severe bottleneck of the Mesola red deer are likely to influence their viability. Catastrophes such as forest fires, floods or diseases are perhaps not very common, but they impose a serious risk on a unique population confined to a small area (cf. Hajji et al. 2007). The effect of establishing two subpopulations with a higher total carrying capacity was assessed because habitat improvements have already led to a considerable increase in population size and fitness (see above), and because this approach would reduce the deer's susceptibility to environmental stochasticity compared to that of a single population. At present, plans of dividing the Mesola population for these very reasons are being carried out, and we aimed at estimating their possible effects.

Demographic parameters for the PVA were chosen according to Mattioli et al. (2003) and unpublished data which can be summarised as follows. The sex ratio (males to females) is 1:1 in calves and 1:1.2 in adults. Females are composed of 15% yearlings and 85% adults, while males are composed of 15% yearlings, 20% subadults and 65% adults. Among adult males, 65% are nine years of age or older. Maximum age is 16 years for males and 17 years for females. The age of the first successful reproduction is 4-5 years in females and nine years in males. Females breed until the age of 15, whereas males stop breeding at 14 years of age. Annual female reproduction rate has been found to be 35-40 calves in 100 females. The proportion of breeding males is about 30%. Annual survival rate for calves and yearlings (irrespective of sex) is 80% and 77.5%, respectively (without management measures). Adult females (2 years or older) have a survival rate of 95%, while subadult males

(2-4 years) show a value of 90%. Adult males (five years and older) also have a survival rate of 95%.

Since management measures led to an increase in fitness parameters (see above), we also included their effect on mortality (survival rates of calves and yearlings increased to 95% and 90%, respectively) to compare population viability with and without these measures.

We applied three different levels of inbreeding depression (none at all, 2.69 diploid lethal equivalents (LE) and 12.3 diploid LE) with a lethal-sublethal ratio of 1:1 (Simmons & Crow 1977, Brook et al. 2002), i.e. 50% of LEs were subject to purging (lethals) and 50% were not much affected by it (sublethals). The value of 2.69 LEs was chosen instead of the classic 3.14 (Ralls et al. 1988) as it was calculated by O'Grady et al. (2006) for the fecundity in the red deer population on the Scottish Isle of Rum based on data from Slate et al. (2000). The higher value of 12.3 LEs is the average value found in a meta-analysis of inbreeding depression across the life-history of 30 species of mammals and birds (O'Grady et al. 2006).

We included two types of catastrophes in our analyses: disease and an environmental catastrophe (forest fire or flood), each with a probability of 0%, 1% or 3%. The severity of these catastrophes was defined as follows: while forest fires or floods cause a decrease in reproduction and survival rate to 75% of the mean, diseases are believed to be more harmful and reduce these two parameters to 50% of their mean. While these values are somewhat arbitrary, they are in concordance with published data on other mammal species (e.g. harbour seals *Phoca vitulina*, Borkenhagen 1993 and lions *Panthera leo*, Packer et al. 1999).

Carrying capacity is difficult to determine, but we chose two different values: 120 and 240. The first value is the one of the latest population estimate (2006), the other one is based on the idea that the Mesola population might be split into two subpopulations of about 120 animals each (see below).

We first ran a simulation with an input file (our 'baseline template' for the sensitivity analysis) for one population with a carrying capacity of 120, neglecting inbreeding depression and catastrophes. This simulation then served as a baseline for comparisons with more complex simulation scenarios.

Since the splitting of the Mesola population has been suggested as a conservation measure (Fico 1998), we also simulated possible effects of this scenario. We based our simulations on two subpopulations both with a carrying capacity of 120 individuals. The first population had an initial size of 120, the second started with 34 (25 female and 9 male) animals (translocated from the first to another area). Establishing several subpopulations from a single endangered population is an appropriate way of reducing the susceptibility to environmental stochasticity (Frankham et al. 2002, Johnsingh et al. 2007). To reduce the inbreeding rate, a mutual exchange (i.e. translocation) of one adult female per year on average between the two subpopulations was incorporated into the calculations as a realistic and affordable amount of artificial gene flow. The simulations for two subpopulations were all run with the same demographic data as outlined above but based on the mortality rates after the management measures. Since catastrophes had a considerable impact on population viability (see Results), we also ran simulations with less severe consequences of catastrophic events (decrease in reproduction and survival rate to 90% and 75% of the mean for fires/floods and diseases, respectively) for the two subpopulations. This mitigation could, for instance, be achieved by vaccinations, veterinary treatment and more effective fire fighting or by additional management measures in the wake of catastrophes aiming at increasing the fitness of surviving animals.

Detailed input data are available from F.E. Zachos upon request. All simulations were run for 100 years with 1000 iterations.

Results

Population genetics

There was no significant linkage disequilibrium between any two loci, and all 20 loci were subsequently used in multi-locus analyses. The MICRO-CHECKER analysis did not yield any positive results for the large allele dropout phenomenon. RT7 was the only locus with a positive result for the possibility of an artificially low heterozygosity due to stutter bands, and four loci (RT7, NVHRT16, INRA111 and VH110) showed signs of null alleles.

Table 1 summarises the results on the genetic variability of the Mesola red deer. Allelic diversity was 5.55, and mean H_O and H_E was 0.50 and 0.61, respectively. H_O was lower than H_E in 17 out of 20 loci, and for three loci (BM4208, NVHRT16 and NVHRT21), this deviation from HWE was statis-

Table 1. Genetic variability of the 25 Mesola red deer analysed. A: number of alleles found, H_O and H_E: observed and expected heterozygosity, P: P-value of test for Hardy-Weinberg-Equilibrium (Bonferroni-corrected significance level: 0.0025, significant deviations from Hardy-Weinberg expectations are denoted by *).

Locus	A	H_O	H_E	P
OarCP26	6	0.28	0.42	0.0463
OarFCB304	3	0.52	0.44	0.7386
MAF35	3	0.07	0.19	0.0374
MAF109	5	0.43	0.56	0.5389
INRA11	7	0.38	0.58	0.0315
BM888	10	0.88	0.78	0.6605
BM4208	8	0.76	0.82	0.0000*
BM4513	9	0.56	0.63	0.2593
NVHRT16	6	0.20	0.66	0.0000*
NVHRT21	9	0.64	0.77	0.0000*
RT1	6	0.83	0.73	0.8441
RT7	7	0.56	0.77	0.0046
INRA121	6	0.52	0.67	0.0242
IDVGA55	3	0.67	0.69	1.0000
BMC1009	3	0.60	0.63	0.8033
BM757	3	0.44	0.52	0.4273
BL42	5	0.57	0.74	0.0142
TGLA53	2	0.32	0.53	0.1058
BM203	5	0.35	0.38	0.1160
VH110	5	0.32	0.60	0.0042

tically significant. The multi-locus test yielded a significant overall heterozygote deficit ($P = 0.0000$). The overall inbreeding coefficient F_{IS} was 0.159.

The proportion of cases where both parents of an individual were present in the sample was zero irrespective of whether zero, one or two incompatibilities were allowed. For only one parent present in the sample, there was one possible parent-offspring pair (out of 150 undirected pairwise comparisons) when no incompatibility was allowed, eight such pairs for one allowed incompatibility and 22 pairs when two incompatibilities were allowed. The two last numbers, however, are potentially inflated by multiple matches for the same individuals. Furthermore, given that these numbers will only refer to real parent-offspring relationships if 1) the mismatches are really due to genotyping errors, 2) the correct but misidentified and unknown alleles happen to be the same as in the other individual, and 3) identical alleles are identical by descent and not only identical by state (length homoplasies), we consider the probability of a bias in our population genetic analysis due to sampling of closely related animals as very low.

$P_{IDstand}$ and P_{IDsib} values differed by between one and eight orders of magnitude (Table 2). If P_{IDsib} was chosen as the conservative boundary, genotyping individuals for the five most informative loci (BM4208, BM888, NVHRT21, RT7 and RT1) already yielded a low probability of identity of 1 individual in 100, which could still be reduced by an order of magnitude by including the three next informative loci (BL42, INRA121 and IDVGA55). The proportion of 2MM-pairs was 0.0167 for the five loci and 0.000 for eight loci. The threshold of 0.005 was first met with seven microsatellites (0.003 for the loci mentioned excluding IDVGA55), but six loci (excluding INRA121 as well) also yielded a good match of 0.0067. Already for five loci, there were no 0MM-pairs, i.e. no two individuals displayed the same genotype at all loci.

Population viability analysis

The comparison between the simulations based on calf and yearling mortality with and without management measures expectedly yielded considerably better prospects for the first case. The mean probability of extinction in nine runs (each possible combination of the three levels of inbreeding depression and catastrophe probability, and with carrying capacity being 120 for a single population) was 62.3% without management measures and 22.2% with management measures (significant difference, Wilcoxon test: $Z = -2.667$, $P = 0.008$). The respective

Table 2. Results of the calculations of probability of identity (P_{ID}). The value given for the standard and sibling P_{ID} is the number of red deer in 100 that could by chance show the same multilocus genotype (i.e. the P_{ID} value multiplied by 100). The values are calculated sequentially starting with the most informative locus (1), then multiplying by the value of the next informative locus (2) and so on until all loci (20) are included.

Locus	$P_{IDstand}(100)$	$P_{IDsib}(100)$
20	4.2×10^{-12}	1.7×10^{-4}
19	5.5×10^{-12}	2.0×10^{-4}
18	1.3×10^{-11}	2.9×10^{-4}
17	3.2×10^{-11}	4.4×10^{-4}
16	8.1×10^{-11}	6.9×10^{-4}
15	2.1×10^{-10}	0.001
14	6.0×10^{-10}	0.002
13	2.1×10^{-9}	0.0035
12	8.5×10^{-9}	0.0064
11	3.0×10^{-8}	0.012
10	1.6×10^{-7}	0.023
9	7.1×10^{-7}	0.047
8	4.1×10^{-6}	0.098
7	2.1×10^{-5}	0.21
6	1.2×10^{-4}	0.44
5	8.3×10^{-4}	1.01
4	0.0061	2.33
3	0.055	5.7
2	0.52	14.16
1	6.5	36.37

Table 3. Impact of levels of inbreeding depression (columns, given as 0, 2.69 and 12.3 diploid lethal equivalents (LE)) and probability of catastrophes (rows, 0%, 1% and 3%) on probability of extinction (PE), stochastic growth rate (GR), final population size among surviving populations (N) and relative final expected heterozygosity (H_E) for a single Mesola population with a carrying capacity of 120 animals. The first value given is based on data before, the second is based on data after management measures were taken. All numbers are mean values of simulations run for 100 years with 1000 iterations.

		0 LE	2.69 LE	12.3 LE
0%	PE	0.095/0.000	0.200/0.000	0.744/0.023
	GR	-0.0119/0.0226	-0.0184/0.0168	-0.0377/-0.0013
	N	43.4/103.0	28.9/96.2	8.5/46.7
	H_E	0.81/0.90	0.80/0.90	0.73/0.87
1%	PE	0.287/0.030	0.547/0.056	0.958/0.289
	GR	-0.0204/0.0106	-0.0314/0.0035	-0.0482/-0.0162
	N	33.7/82.7	19.4/68.0	5.6/26.7
	H_E	0.78/0.87	0.75/0.85	0.73/0.83
3%	PE	0.844/0.327	0.934/0.445	0.992/0.830
	GR	-0.0469/-0.0145	-0.0534/-0.0211	-0.0625/-0.0393
	N	16.2/47.3	13.7/36.4	3.3/16.0
	H_E	0.70/0.79	0.70/0.79	0.52/0.78

values for final expected heterozygosity (relative to generation 1 whose heterozygosity is defined as 100%) were 72.5% and 84.1% ($Z=-2.700$, $P=0.007$). The mean stochastic (simulated) growth rate was -0.0368 without vs -0.0043 with management measures ($Z=-2.667$, $P=0.008$), and the final size of non-extinct populations was on average 19.2 vs 58.1 with and without management measures, respectively ($Z=-2.667$, $P=0.008$).

Both inbreeding depression and catastrophes had a large impact on the population's viability parameters (Table 3). Prospects were especially bleak with a combination of high inbreeding depression and a high probability of the occurrence of catastrophes. However, the buffering effect of the management measures was considerable. Apart from the significantly different means given above, it is of particular interest that these measures were able to largely compensate for the consequences of inbreeding, and that negative stochastic growth rates were only found for worst-case scenarios for inbreeding depression and/or catastrophes, whereas without management measures, they were never positive. Splitting of the Mesola population into two subpopulations further mitigated the negative consequences of inbreeding depression and catastrophes (Table 4). All four parameters, probability of extinction, stochastic growth rate, final population size and relative expected heterozygosity, were statistically significantly more favourable under the two-subpopulations-scenarios than they were when assuming only one population (Wilcoxon tests, PE: means 22.2% vs 15.7%, $Z=-2.366$, $P=0.018$; GR: means -0.0043 vs -0.0017, $Z=-2.312$, $P=0.021$; N: means 58.1 vs 105.1, $Z=-2.547$, $P=0.011$; H_E: means 84.1% vs 87.9%, $Z=-2.552$, $P=0.011$). Under the worst-case scenario (12.3 lethal equivalents and a 3% probability for each of the two catastrophes), however, this difference was only obvious for the probability of extinction while for the other three parameters, the values for two subpopulations were actually worse than for a single population (see Tables 3 and 4).

Mitigation of the impact of catastrophic events, as compared for the two-subpopulations scenarios, confirmed that catastrophes had a very high potential of reducing population viability. With a less severe reduction of survival and reproduction to 90% and 75% of their means for the two catastrophe types, the two subpopulations performed much better than under the assumption that these fitness parameters are reduced to 75% and 50%, respectively (see Table 4). Again, these differences were statistically significant for all four parameters (Wilcoxon tests, PE: means 23.4% vs 4.2%, $Z=-2.201$, $P=0.028$; GR: means -0.0098 vs 0.0048, $Z=-2.207$, $P=0.027$; N: means 78.7 vs 124.1, $Z=-2.201$, $P=0.028$; H_E: means 85.2% vs 90.5%, $Z=-2.214$, $P=0.027$), and this also holds for the worst-case scenario: extinction probability was reduced by 2/3,

Table 4.. Impact of inbreeding depression and catastrophes (the same levels as in Table 3) on viability parameters of the Mesola red deer based on two subpopulations (for details see text). PE: probability of extinction, GR: stochastic growth rate, N: final population size among surviving populations, H_E: relative final expected heterozygosity. The second value is based on less severe effects of catastrophic events (see text). All numbers are mean values of simulations run for 100 years with 1000 iterations.

		0 LE	2.69 LE	12.3 LE
0%	PE	0	0	0.005
	GR	0.025	0.02	-0.001
	N	203.5	190.1	80.5
	H_E	0.94	0.94	0.92
1%	PE	0.001/0.000	0.005/0.000	0.175/0.032
	GR	0.016/0.022	0.009/0.015	-0.016/-0.007
	N	163.3/194.9	135.7/173.2	38.0/58.2
	H_E	0.92/0.94	0.91/0.93	0.86/0.89
3%	PE	0.161/0.000	0.277/0.006	0.787/0.213
	GR	-0.009/0.013	-0.018/0.006	-0.041/-0.020
	N	70.8/161.7	49.3/127.2	14.9/29.6
	H_E	0.83/0.92	0.82/0.91	0.77/0.84

population decline was halved and final population size was doubled.

Discussion

Our analyses yielded bleak prospects for the Mesola population with high probabilities of extinction, but we could also show that management measures, in particular the establishment of two subpopulations with artificial gene flow among them, and mitigation of the effect of catastrophic events, are capable of significantly reducing the risk of extinction and the critical decay of genetic variability.

Low genetic diversity in the Mesola red deer has been confirmed based on a much larger number of microsatellite loci than previously analysed. Expected heterozygosity is even lower than that of the long-term isolated and bottlenecked Sardinian red deer *C. e. corsicanus* (0.61 vs 0.66, Hmwe et al. 2006) and that of a severely inbred small isolate in northern Germany (again 0.61 vs 0.66, Zachos et al. 2007). The overall heterozygote deficit indicates that inbreeding is a relevant factor in the Mesola population, although the inbreeding coefficient (F_{IS}) of 0.159 was not exceedingly high in the light of the demographic history of the population. Still, it was higher than expected under half-sib mating and higher than the inbreeding coefficient of the aforementioned red deer from northern Germany that already suffer severe inbreeding depression (Zachos et al. 2007). Moreover, inbreeding is always a relative measure with F_{IS} being based on deviations from random mating. Random mating within small and inbred populations where all animals are more or less closely related may well lead to an increase of pedigree inbreeding (and inbreeding depression) without raising F_{IS} (Keller & Waller 2002).

As mentioned before, a genetic screening of the Mesola red deer has been demanded. Yet, genetic analyses are expensive and conservation budgets are limited. The results of our study indicate that 5-7 microsatellite loci are sufficient to resolve individuals with a reasonable amount of certainty so that a future genetic screening of the Mesola red deer (to document the amount of drift and inbreeding in one or more subpopulations) can be carried out with this comparatively low number of loci. At least nine loci are needed to resolve individuals of the similarly small and isolated Apennine brown bear *Ursus arctos* population (Lorenzini et al. 2004).

The average effective population size of the Mesola population has been estimated to be about 15 individuals, and accordingly, an increase in inbreeding of 3.3% per generation has been calculated (Lorenzini et al. 1998). This value is more than three times higher than the threshold of 1% up to which natural selection is believed to overpower the fixation of deleterious alleles (Franklin 1980, Soulé 1980). Therefore, deleterious alleles become effectively neutral and governed by drift rather than selection which will increase the mutational load of the population. As a result, its fitness may decline over time (Hedrick & Kalinowski 2000, Reed & Frankham 2003) and the population may decrease in size, which will lead to even more detrimental alleles being subject to drift and random fixation. This mutational meltdown (Lynch et al. 1995) may eventually threaten the population with extinction. It is also noteworthy that in populations with long-term low effective population size (such as Mesola), purging may have acted against inbreeding depression. This, however, may only lead to a time lag until inbreeding accumulates and increases extinction risk (O'Grady et al. 2006) as purging is usually not able to effectively compensate for inbreeding (Frankham et al. 2001, Reed et al. 2003). The fact that the fitness of the Mesola red deer has been increased through management measures may thus not be taken as evidence for the present (or future) absence of inbreeding depression. It has recently been shown for naked mole-rats *Heterocephalus glaber* that inbreeding can lead to increased disease susceptibility even in the absence of other inbreeding depression symptoms (Ross-Gillespie et al. 2007).

Our PVA analyses confirm that given the demographic data and history of the Mesola red deer, inbreeding depression has a considerable impact on extinction risk and other viability parameters. The difference between 12.3 and 2.69 LEs was much more distinct than the one between 2.69 and 0 LEs. Unfortunately, 12.3 LEs are probably much more realistic than 2.69 as the latter value was calculated taking into account only life-time breeding success, whereas the higher number of 12.3 was estimated across the life-history and thus encompasses more fitness parameters than breeding success alone (Slate et al. 2000, O'Grady et al. 2006). Moreover, the value of 2.69 LEs refers to the red deer population on the Isle of Rum off Scotland's coast, which is much bigger and less affected by inbreeding than the Mesola population.

It is one goal in the management of endangered populations to retain 90% of their genetic diversity for 100 years (Soulé et al. 1986, Frankham et al. 2002). In our simulations, genetic diversity (as measured by expected heterozygosity) was considerably higher after implementation of management measures, and in particular under the scenario of two subpopulations (the lowest relative H_E value being 77% in the latter case, which is equivalent to an absolute H_E of $0.61 \times 0.77 = 0.47$). Given the very low initial genetic diversity of these red deer (0.61) and taking into account that heterozygosity is correlated with fitness in many cases (Reed & Frankham 2003), management actions should explicitly aim at creating conditions under which as little heterozygosity as possible will be lost in the future. Artificially increasing genetic diversity through introduction of non-related red deer (as was done with the Florida panther *Puma concolor coryi*, Pimm et al. 2006) should be avoided as this would mean polluting the genetic integrity of this unique population. Should inbreeding depression become an obviously decisive factor, then the final options could be to try to artificially raise the effective population size by equalising family sizes, removing long-term reproductive males, and exchanging more individuals between subpopulations.

With its unique mitochondrial haplotype found nowhere else in Europe and its significant genetic differentiation from other red deer (Lorenzini et al. 2005, Hmwe et al. 2006), the Mesola population clearly meets the criteria of being treated as a separate management unit (Moritz 1994). Our sensitivity analyses have shown that there are alternatives to the introduction of animals of which the establishment of two subpopulations (with artificial gene flow) and the mitigation of the impact of potential catastrophes seem to be particularly fruitful. By implementing these measures, loss of heterozygosity and probability of extinction can be further reduced.

It is a general problem of population viability analyses that for some of the required input parameters no actual field data are known. Thanks to the detailed survey of the Mesola population, we had a large body of demographic and life-history data for these animals, and even the values chosen for carrying capacity and inbreeding depression are derived from actual studies or available data. Variation of catastrophic impacts showed that this parameter was crucial as was the probability of the occurrence of catastrophic events. This is the strength of sensitivity analyses, applying a range of values for uncertain input parameters and getting an idea of the relative impact of different parameters on population viability (Miller & Lacy 2005). Therefore, the most important results of our simulations are the relative ones: relative increase in viability with 1) management measures (reduction of competition with fallow deer, winter feeding and habitat improvement), 2) a metapopulation framework, and 3) reduction of the impact of catastrophes. These three points led to significantly better prospects in our simulations and were shown to be valuable tools for the future management and conservation of the Mesola population. Given that these deer are a low-performance population, it might be assumed that the foundation of another subpopulation in good habitat will show better demographic characteristics, in particular a higher birth rate, faster sexual maturation and a higher proportion of juveniles. This would additionally ameliorate the population's poor prospects, but if and to what extent this holds can only be analysed after two or more subpopulations have been established and monitored for some generations. Since the estimated effective population size of 15 is only about one third of 50, which is generally regarded as a minimum number to avoid inbreeding depression (Frankham et al. 2002), the Mesola population may still face serious problems in the future even if the consequences of environmental stochasticity can be buffered by the establishment of two subpopulations.

The Mesola red deer are the last remaining genetic legacy from the formerly widespread red deer population on the Italian mainland. In addition to the large body of data from the long-term population survey, local authorities now have further information at their disposal to realise the recommendations of the Mesola report (Fico 1998), and to estimate the costs involved in this. Further, our study highlights the positive effects of population subdivision, a conservation measure that is also being carried out in other species (e.g. the Asiatic lion *Panthera leo persica*, Johnsingh et al. 2007) and that may turn out to be of great value in conservation biology in general.

Acknowledgements - the authors wish to express their gratitude to three anonymous referees whose comments on an earlier version of the manuscript have greatly improved this study.

References

Allendorf, F.W. & Luikart, G. 2007: Conservation and the Genetics of Populations. - Blackwell, Malden, Oxford, Carlton, 642 pp.

Bonnet, A., Thévenon, S., Maudet, F. & Maillard, J.C. 2002: Efficiency of semi-automated fluorescent multiplex PCRs with 11 microsatellite markers for genetic studies of deer populations. - Animal Genetics 33: 343-350.

Borkenhagen, P. 1993: Atlas der Säugetiere Schleswig-Holsteins. - Landesamt für Naturschutz und Landschaftspflege Schleswig-Holstein, Kiel, 131 pp. (In German).

Brook, B.W., O'Grady, J.J., Chapman, A.P., Burgman, M.A., Akcakaya, H.R. & Frankham, R. 2000: Predictive accuracy of population viability analysis in conservation biology. - Nature 404: 385-387.

Brook, B.W., Tonkyn, D.W., O'Grady, J.J. & Frankham, R. 2002: Contribution of inbreeding to extinction risk in threatened species. - Conservation Ecology 6: 16 (online). Available at: http://www.consecol.org/vol6/iss1/art16

Estonba, A., Solís, A., Iriondo, M., Sanz-Martín, M.J., Pérez-Suárez, G., Markov, G. & Palacios, F. 2006: The genetic distinctiveness of the three Iberian hare species: *Lepus europaeus*, *L. granatensis*, and *L. castroviejoi*. - Mammalian Biology 71: 52-59.

Evett, I. & Weir, B. 1998: Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists. - Sinauer, Sunderland, 278 pp.

Ewen, K.R., Bahlo, M., Treloar, S.A., Levinson, D.F., Mowry, B., Barlow, J.W. & Foote, S.J. 2000: Identification and analysis of error types in high-throughput genotyping. - American Journal of Human Genetics 67: 727-736.

Fico, R. (Ed.) 1998: Rapporto finale sul Progetto Cervo della Mesola. - Istituto Zooprofilattico Sperimentale, Teramo, Italy. (In Italian).

Frankham, R., Ballou, J.D. & Briscoe, D.A. 2002: Introduction to Conservation Genetics. - Cambridge University Press, Cambridge, New York, 617 pp.

Frankham, R., Gilligan, D.M., Morris, D. & Briscoe, D.A. 2001: Inbreeding and extinction: effects of purging. - Conservation Genetics 2: 279-285.

Franklin, J.R. 1980: Evolutionary change in small populations. - In: Soulé, M.E. & Wilcox, B.A. (Eds.); Conservation biology. An evolutionary-ecological perspective Sinauer, Sunderland, pp. 135-149.

Geist, V. 1987: On the evolution of optical signals in deer. A preliminary analysis. - In: Wemmer, C.M. (Ed.); Biology and management of the Cervidae. Smithsonian Institution Press, Washington, pp. 235-255.

Goudet, J. 1995: FSTAT (Version 1.2): A Computer Program to calculate F-Statistics. - Journal of Heredity 86: 485-486.

Hajji, G.M., Zachos, F.E., Charfi-Cheikrouha, F. & Hartl, G.B. 2007: Conservation genetics of the imperilled Barbary red deer in Tunisia. - Animal Conservation 10: 229-235.

Hedrick, P.W. & Kalinowski, S.T. 2000: Inbreeding depression in conservation biology. - Annual Review of Ecology and Systematics 31: 139-162.

Hmwe, S.S., Zachos, F.E., Eckert, I., Lorenzini, R., Fico, R. & Hartl, G.B. 2006: Conservation genetics of the endangered red deer from Sardinia and Mesola with further remarks on the phylogeography of *Cervus elaphus corsicanus*. - Biological Journal of the Linnean Society 88: 691-701.

Hosack, D.A., Miller, P.S., Hervert, J.J. & Lacy, R.C. 2002: A population viability analysis for the endangered Sonoran pronghorn, *Antilocapra americana sonoriensis*. - Mammalia 66: 207-229.

Johnsingh, A.J.T., Goyal, S.P. & Qureshi, Q. 2007: Preparations for the reintroduction of Asiatic lion *Panthera leo persica* into Kuno Wildlife Sanctuary, Madhya Pradesh, India. - Oryx 41: 93-96.

Keller, L.F. & Waller, D.M. 2002: Inbreeding effects in wild populations. - Trends in Ecology and Evolution 17: 230-241.

Koskinen, M.T., Hirvonen, H., Landry, P-A. & Primmer, C.R. 2004: The benefits of increasing the number of microsatellites utilized in genetic population studies: an empirical perspective. - Hereditas 141: 61-67.

Kuehn, R., Schroeder, W., Pirchner, F. & Rottmann, O. 2003: Genetic diversity, gene flow and drift in Bavarian red deer populations (*Cervus elaphus*). - Conservation Genetics 4: 157-166.

Lacy, R.C., Borbat, M. & Pollak, J.P. 2005: VORTEX: A stochastic simulation of the extinction process. Version 9.50. - Chicago Zoological Society, Brookfield, Illinois, USA. Available at: http://www.vortex9.org/vortex.html

Lorenzini, R., Fico, R. & Mattioli, S. 2005: Mitochondrial DNA evidence for a genetic distinction of the native red deer of Mesola, northern Italy, from the Alpine populations and the Sardinian subspecies. - Mammalian Biology 70: 187-198.

Lorenzini, R., Lovari, S. & Masseti, M. 2002: The rediscovery of the Italian roe deer: genetic differentiation and management implications. - Italian Journal of Zoology 69: 367-379.

Lorenzini, R., Mattioli, S. & Fico, R. 1998: Allozyme variation in native red deer *Cervus elaphus* of Mesola Wood, northern Italy: implications for conservation. - Acta Theriologica Suppl. 5: 63-74.

Lorenzini, R., Posillico, M., Lovari, S. & Petrella, A. 2004: Non-invasive genotyping of the endangered Apennine brown bear: a case study not to let one's hair down. - Animal Conservation 7: 199-209.

Lynch, M., Conery, J. & Burger, R. 1995: Mutation meltdowns in sexual populations. - Evolution 49: 1067-1088.

Mattioli, S. 1990: Red deer in the Italian peninsula with particular reference to the Po delta population. - Deer 8: 95-98.

Mattioli, S. 1993: Antler conformation in red deer of the Mesola Wood, northern Italy. - Acta Theriologica 38: 443-450.

Mattioli, S., Fico, R., Lorenzini, R. & Nobili, G. 2003: Mesola red deer: physical characteristics, population dynamics and conservation perspectives. - Hystrix, Italian Journal of Mammalogy 14: 87-94.

Miller, P.S. & Lacy, R.C. 2005: VORTEX: A stochastic simulation of the extinction process. Version 9.50 Users's Manual. - Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, Minnesota, USA, 149 pp.

Moritz, C. 1994: Defining 'Evolutionarily Significant Units' for conservation. - Trends in Ecology and Evolution 9: 373-375.

O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W. & Frankham, R. 2006: Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. - Biological Conservation 133: 42-51.

Packer, C., Altizer, S., Appel, M., Brown, E., Martenson, J., O'Brien, S.J., Roelke-Parker, M., Hofmann-Lehmann, R. & Lutz, H. 1999: Viruses of the Serengeti: Patterns of infection and mortality in African lions. - Journal of Animal Ecology 68: 1161-1178.

Paetkau, D. 2003: An empirical exploration of data quality in DNA-based population inventories. - Molecular Ecology 12: 1375-1387.

Paetkau, D. & Strobeck, C. 1994: Microsatellite analysis of genetic variation in black bear populations. - Molecular Ecology 3: 489-495.

Pemberton, J.M., Slate, J., Bancroft, D.R. & Barrett, J.A. 1995: Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. - Molecular Ecology 4: 249-252.

Pimm, S.L., Dollar, L. & Bass, O.L. Jr. 2006: The genetic rescue of the Florida panther. - Animal Conservation 9: 115-122.

Ralls, K., Ballou, J.D. & Templeton, A. 1988: Estimates of lethal equivalents and the cost of inbreeding in mammals. - Conservation Biology 2: 185-193.

Raymond, M. & Rousset, F. 1995: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. - Journal of Heredity 86: 248-249.

Redeker, S., Andersen, L.W., Pertoldi, C., Madsen, A.B., Jensen, T.S. & Jørgensen, J.M. 2006: Genetic structure, habitat fragmentation and bottlenecks in Danish bank voles (*Clethrionomys glareolus*). - Mammalian Biology 71: 144-158.

Reed, D.H. & Frankham, R. 2003: Correlation between fitness and genetic diversity. - Conservation Biology 17: 230-237.

Reed, D.H., Lowe, E.H., Briscoe, D.A. & Frankham, R. 2003: Inbreeding and extinction: effects of rate of inbreeding. - Conservation Genetics 4: 405-410.

Rice, W.R. 1989: Analyzing tables of statistical tests. - Evolution 43: 223-225.

Ross-Gillespie, A., O'Riain, M.J. & Keller, L.F. 2007: Viral epizootic reveals inbreeding depression in a habitually inbreeding mammal. - Evolution 61: 2268-2273.

Schneider, S., Roessli, D. & Excoffier, L. 2000: Arlequin ver 2.000: A software for population genetics data analysis. - Genetics and Biometry Laboratory, University of Geneva, Switzerland. Available at: http://cmpg.unibe.ch/software/arlequin3/

Simmons, M.J. & Crow, J.F. 1977: Mutations affecting fitness in Drosophila populations. - Annual Review of Genetics 11: 47-78.

Slate, J., Kruuk, L.E.B., Marshall, T.C., Pemberton, J.M. & Clutton-Brock, T.H. 2000: Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). - Proceedings of the Royal Society London B 267: 1657-1662.

Slate, J. & Pemberton, J.M. 2002: Comparing molecular measures for detecting inbreeding depression. - Journal of Evolutionary Biology 15: 20-31.

Soulé, M.E. 1980: Threshold for survival. Maintaining fitness and evolutionary potential. - In: Soulé, M.E.. & Wilcox, B.A. (Eds.); Conservation biology. An evolutionary-ecological perspective. Sinauer, Sunderland, pp. 151-169.

Soulé, M.E., Gilpin, M., Conway, W. & Foose, T. 1986: The millenium ark: how long a voyage, how many staterooms, how many passengers? - Zoo Biology 5: 101-113.

Valière, N. 2002: GIMLET: a computer program for analysing genetic individual identification data. - Molecular Ecology Notes 2: 377-379.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004: MICRO-CHECKER (Version 2.2.3): Software for identifying and correcting genotyping errors in microsatellite data. - Molecular Ecology Notes 4: 535-538.

Waits, L.P., Luikart, G. & Taberlet, P. 2001: Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. - Molecular Ecology 10: 249-256.

Wattier, R., Engel, C.R., Saumitou-Laprade, P. & Valero, M. 1998: Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). - Molecular Ecology 7: 1569-1573.

Wisdom, M.J. & Mills, L.S. 1997: Sensitivity analysis to guide population recovery: prairie chickens as an example. - Journal of Wildlife Management 61: 302-312.

Zachos, F.E., Althoff, C., v. Steynitz, Y., Eckert, I. & Hartl, G.B. 2007: Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. - European Journal of Wildlife Research 53: 61-67.

Zachos, F., Hartl, G.B., Apollonio, M. & Reutershan, T. 2003: On the phylogeographic origin of the Corsican red deer (*Cervus elaphus corsicanus*): evidence from microsatellites and mitochondrial DNA. - Mammalian Biology 68: 284-298.

Zachos, F.E., Hmwe, S.S. & Hartl, G.B. 2006: Biochemical and DNA markers yield strikingly different results regarding variability and differentiation of roe deer (*Capreolus capreolus*, Artiodactyla: Cervidae) populations from northern Germany. - Journal of Zoological Systematics and Evolutionary Research 44: 167-174.