Ital. J. Zool., 66: 351-353 (1999)

Genetic bottleneck in the threatened western population of European mink *Mustela lutreola*

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ABSTRACT

The western population of European mink *Mustela lutreola* has recently suffered a severe decline in over more than 50% of its range, probably disappearing from Brittany in 1992 and from Pays de Loire in 1997. Allozyme electrophoresis on 38 presumptive loci showed that the proportion of polymorphic loci reached only 10.5% at $P < 0.01$ level in the European mink. Observed heterozygosity averaged H_o = 0.02 and expected heterozygosity H_E = 0.038 but the F_{IS} index reached 0.48, revealing a considerable deficit. The low genetic variability might be due to previous bot tlenecks. Demographic depletion in *Mustela lutreola* resulted in a loss of genetic diversity emphasising that reproductive exchanges were altered and worsening the risk of extinction of this vulnerable population.

KEY-WORDS: Heterozygosity - Population genetics - Mustelid - Mammals - Endangered species.

ACKNOWLEDGEMENTS

I would like to thank D. Le Jacques and A. M. O'Donovan for their assistance, and I express my appreciation to C. Maizeret and Fédération Départementale des Chasseurs (FDC) for providing tissue samples from Aquitaine. Prof. S. Lovari provided helpful criticism on the manuscript.

(Received 15 June 1999 - Accepted 10 September 1999)

INTRODUCTION

Population depletion results in a loss of genetic diversity. Small populations tend to lose their genetic variability over time and inbreeding is demonstrated to increase the risk of extinction because it depresses reproductive fitness (Jimenez *et al.*, 1994; Bouzat *et al.*, 1998). Due to the lack of previous data, the fact that a population has suffered a genetic bottleneck is rarely supported, and numerous studies only assumed that a low genetic diversity results from past bottlenecks (Bonnel & Selander, 1974; O'Brien *et al.*, 1983).

The range of the European mink *Mustela lutreola* Linnaeus, 1761 was fragmented into eastern (from Ural to the Black Sea) and western populations (Youngman, 1982). While its western population range spread from Brittany to Spain up until 1984, the species has recently disappeared in 1992 from Brittany and probably in 1997 from Vendée (last data: Isac River, 47°34N-2°50W, Lodé, 1992 Lay River, 46°48N-0°57W) (Fig. 1). The actual range of the mink western population is now restricted to south western France and northern Spain (Ruiz-Olmo & Palazon, 1996; Maizeret *et al.*, 1998). The European mink is now regarded as one of the most endangered species in Europe (Schreiber *et al.* 1989).

This severe demographic decline in over 50% of the range may have affected the genetic diversity of the *Mustela lutreola* western population. Documenting population genetics is an important concern for designing management strategies for the recovery of endangered species. Until now, no studies on allozymic variation in the European mink have been documented. The aim of this study is to investigate the genetic heterozygosity inferred from allozymic variations in the European mink western population.

MATERIALS AND METHODS

Tissue samples were opportunistically collected from 12 road-killed European minks from Vendée to the Pyrénées (9 provided by Maizeret, 2 by FDC Gironde, and an individual found dead in Vendée) between 1993 and 1997. One gram of muscle tissue was removed from the back limb and homogenised. Samples were centrifuged for 15 minutes at 10,000g and extracts were prepared for horizontal starch gel electrophoresis with three buffer systems (TC6, TC8 and TEB) at 4° for 3 to 5 hours. Electrophoretic procedures were used following Pasteur *et al.* (1987), Murphy *et al.* (1990), Rothe (1994) and 38 presumptive gene loci encoding 24 enzymes were scored [Aat-1 and Aat-2 (E.C. 2.6.1.1), Aco-1 and Aco-2 (4.2.1.3), Ada (3.5.4.4), Ak (2.7.4.3), Ck-1 and Ck-2 (2.7.3.2), Ddh-1 and Ddh-2 (1.8.1.4), Est-1 and Est-2 (3.1.1.1), Fumh (4.2.1.2), Gly2dh (1.1.1.29), G6pdh (1.1.1.49), Gpi (5.3.1.9), Hk-1, Hk-2 and Hk-3 (2.7.1.1), Idh-1 and Idh-2 (1.1.1.42), Ldh-1 and Ldh-2 (1.1.1.27), Mdh-1 and Mdh-2 (1.1.1.37), Me-1 and Me2 (1.1.1.40), Mpi (5.3.1.8), Pep-1 and Pep-2 (3.4.11.1), Pgdh (1.1.1.44), Pgm-2 (2.7.5.1), Pnp (2.4.2.1), Sdh (1.1.1.14), Sod (1.15.1.1), Tpi (5.3.1.1), and two non specific proteins]. Observed (H_o) and expected (H_E) average heterozygosity (Nei, 1978) was estimated using Genetix software (Laboratory Genome and Population). The estimate of heterozygote deficiency was based on the F_{IS} index as in Weir & Cockerham (1984).

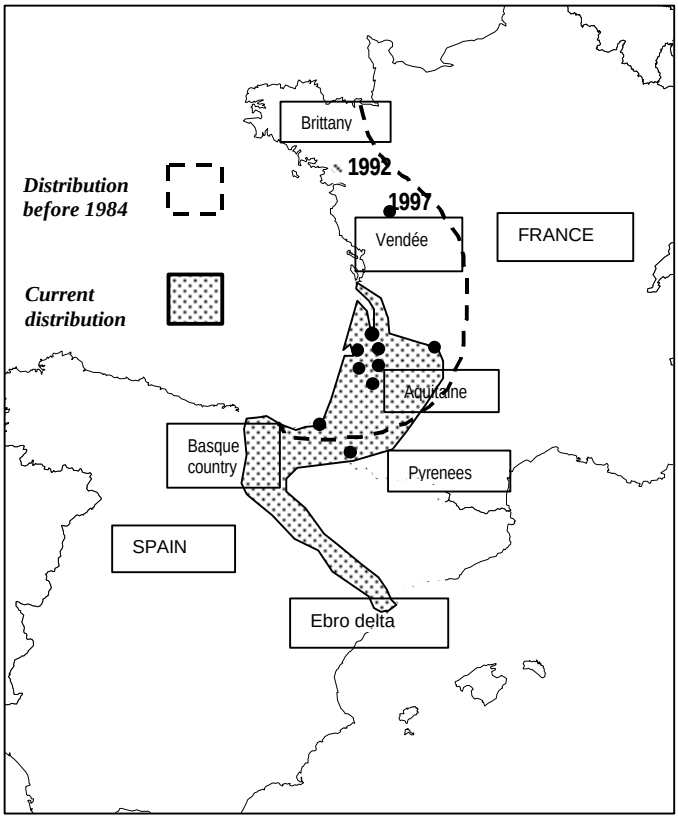

Fig. 1 - Range of *Mustela lutreola* western population (from Ruiz-Olmo & Palazon, 1996, and Maizeret *et al.*, 1998) and sampled localities.

TABLE I - *Observed and expected heterozygosity in* Mustela lutreola *from western France.*

Loci	H_O	H_E
Est-2	0.09	0.37
Mdh-1	0.08	0.08
Me-1	0.10	0.48
Pnp	0.50	0.52
Mean H	0.020	0.038
Sd	0.084	0.126

RESULTS AND DISCUSSION

Assessing 38 presumptive loci, only four loci were found to be polymorphic at $P < 0.01$ level with two alleles.

Observed heterozygosity in the *Mustela lutreola* western population averaged $H_O = 0.020$. (Table I) but one locus (Pnp) contributed 65% of the whole heterozygosity. Average expected heterozygosity for small sample was $H_E = 0.038$. The estimate of the F_{IS} index reached 0.48. This result revealed a clear heterozygote deficiency. Thus, the European mink western population has lost almost a half of its previous heterozygosity.

Within the general context of the decline of European mink populations, these results raise considerable issues for species conservation.

First, the *Mustela lutreola* population from western France exhibited low genetic variability which is clearly suggestive of previous population bottlenecks (Nei *et al.*, 1975). According to Nei's prediction (1978), a genetic bottleneck chiefly affects the number of polymorphic loci by reducing allelic diversity. In *Mustela lutreola*, only 10.5% of loci were shown to be polymorphic whereas the polymorphism reached 26.5% in the polecat population from western France (Lodé, 1998).

Second, the European mink western population displayed a lower level of heterozygosity than did the polecat population from western France and showed a significant heterozygote deficiency. Mammalian species have been previously suspected to have only low heterozygosity levels (Simonsen, 1982; Wooten & Smith, 1985). However, this assumption was not supported later and levels of heterozygosity reported in numerous mustelids varied from 0.17 in *Martes americana* (Mitton & Raphael 1990), 0.064 in *Mustela nivalis* (Hartl *et al.*, 1988), 0.057 in *Mustela putorius* (Lodé 1998), 0.046 in *Enhydra lutris* (Lidicker & MacCollum, 1997) to 0.032 in *Lontra canadensis* (Serfass *et al.*, 1998). Nevertheless, it may be assumed that a long-time at low densities can also affect heterozygosity and the European mink has never been abundant in France (Bree & Saint Girons, 1966). A heterozygosity deficiency affected the *Mustela lutreola* western population revealing that the reproductive exchanges were altered. Inbreeding depression has been demonstrated even in natural conditions (Jimenez *et al.*, 1994) and could considerably increase the risk of extinction in vulnerable populations (Mills & Smouse, 1994).

Preserving genetically distinct populations is a fundamental concern for biodiversity (Frankel & Soulé, 1981). Small isolated populations are more exposed to extinction than others (Frankham & Ralls, 1998). The severe decline in *Mustela lutreola* clearly results in a loss of genetic diversity. Despite the dispersal capacity of mink, evidence from genetics emphasised that reproductive exchanges within population were considerably deteriorated. Further, the lack of genetic diversity due to a population bottleneck is likely to be associated with altered fitness (Bouzat *et al.*, 1998). A program for the conservation of *Mustela lutreola* western population is urgently needed, and should consider both the genetic characteristics of the western population and restoring reproductive exchanges.

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