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Genetic heterozygosity in polecat *Mustela putorius* populations from Western France

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Allozymic variations were investigated in 49 European polecats *Mustela putorius* from western France by starch gel electrophoresis. Out of 31 surveyed loci, eight (25.8%) were shown polymorphic and observed heterozygosity averaged 0.057. Deviations from Hardy-Weinberg equilibrium and heterozygote deficiency suggest that populations were not in panmixia. Heterozygotes for two loci or more totalled 42.9% of individuals. Thus, although carnivores were previously considered as less variable, polecat populations from western France showed a high genetic variability.

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Most of Mustelidae are generally regarded as relatively little specialised carnivores although they constitute a basic component in numerous ecosystems (cf. GITTLEMAN 1996). The European polecat *Mustela putorius* L. 1758 is a typical medium-sized mustelid, which shows a real eclecticism in its di-etary habits. Polecats mainly exploit rodent and anuran prey but are found in a great diversity of habitats (LODÉ 1997) such as woodland, deciduous and mixed forest, farmland, marsh, river banks, Russian steppe, and even rubbish tips. The ex-ploitation of such distinct habitats entails that pole-cat populations suffer numerous disparate constraints.

Genetic variability allows populations to respond to changing environmental constraints. In the long term, the level of genetic diversity contributes to the adaptive potential of species (FRANKEL and SOULÉ 1981; ALLENDORF and LEARY 1986). Surprisingly, evidence of genetic heterozygosity level in mustelids remains equivocal. When MITTON and RAPHAEL (1990) found 0.17% of average heterozygosity investigating 20 loci in 10 American martens *Martes americana*, SIMONSEN (1982) noticed no variation for 21 loci in 121 Stone martens *Martes foina* and in 24 polecats from Denmark.

Although mammals are generally considered as showing low variability (WOOTEN and SMITH 1985), it could be however, hypothesised that the polecat's ability to face up with various environ-ments may be associated with a certain genetic vari-ability. Hence, I investigated allozyme variability in 49 polecats from western France to assess the lev-els of heterozygosity. Results are discussed in the general context of the assumption of the low ge-netic diversity in mammals.

MATERIAL AND METHODS

Muscle tissue samples were obtained from 49 road-killed adult polecats (18 females and 31 males) opportunistically collected between 1995 and 1997 from western France (Brittany and Pays de Loire region, authorisation DPN 95-97).The sample was uniformly collected in the study area (five individuals in central Brittany, five in the Vannetais area, two near the river Vilaine, three in the north of Brière, two near Guérande area, seven in south Western Brière, eight in Northern Loire-Atlantique, five in Maine-et-Loire, five in Southern Loire-Atlantique and seven in Vendée). The species is common in western France and locally reaches a mean density of 0.8 individual per 1km² but populations suffer a decline in Brittany (LODÉ 1993). Samples were homogenised in equal volume of distilled water and centrifuged at 10000g for 15 minutes at 4°C. Each gel included some samples of control individuals as indicator of relative migration. The homogenates were applied on filter paper, which were removed after 15 minutes of electrophoresis. Then, proteins were separated at approximately 4°C for 3-5 hours. A total of 31 presumptive structural gene loci were successfully investigated by horizontal starch (Sigma) gel electrophoresis using two continuous buffer systems (Tris-Citrate pH6 and Tris-EDTA-Borate pH8). Slices were stained for enzymes following PASTEUR et al. (1987) and MURPHY et al (1990). The protein systems examined were AAT-1 and AAT-2 (E.C. 2.6.1.1), 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), FBP (3.1.3.11), FUMH (4.2.1.2), Gly2DH (1.1.1.29), G6PDH (1.1.1.49), GPI (5.3.1.9), LDH-1 and LDH-2 (1.1.1.27), MDH-1 and MDH-2 (1.1.1.37), ME-1 and

ME-2 (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), SOD (1.15.1.1), TPI (5.3.1.1), and two non specific proteins. Electromorphs were presumed to have a simple genetic basis and alleles were scored in decreasing order of mobility.

Genotypic frequencies at each locus were tested for fit to Hardy-Weinberg equilibrium using Computer program GENEPOP (version 3.1b) (RAYMOND and ROUSSET 1995). Expected numbers of homozygotes or heterozygotes were computed using Levene's correction and F-statistics were estimated as in WEIR and COCKERHAM (1984). The proportion of effective number of alleles resulted from the geometric mean of all n_e's with $n_e = 1/\sum pi^2$. Differences in mean observed heterozygosity levels between males and females were appreciated by the Welch's approximate t test.

RESULTS AND DISCUSSION

Allozyme variations

Out of 31 scored loci, eight (25.8%) were found polymorphic at the 0.05 level showing two alleles per locus (Table 1). The total effective number of alleles was $n_e = 1.26$ over all the loci. The mean number of alleles per polymorphic locus ranged from 1.14 (G6PDH) to 1.99 (PNP). Such variability was not previously mentioned in an European mustelid (WAYNE and KOEPFLI 1996). Although this polymorphism remained lower than that scored in *Martes americana* (33%, MITTON and RAPHAEL 1990), this rate surpassed the polymorphism recorded in Californian sea otter *Enhydra lutris* (19.4%, LIDICKER and MCCOLLUM 1997) or in endangered black footed ferret *Mustela nigripes* (4.3%, O'BRIEN et al. 1989). Nevertheless, four loci were weakly polymorphic with one allele reaching an allelic proportion equal to or less than 0.1 (EST-2, G6PDH, PEP-2 and PGM-2). Genotypic frequencies for six polymorphic loci were in Hardy-Weinberg equilibrium whereas only two loci (ME-1 and PEP-2) showed significantly deviations from expected equilibrium (Table 1).

Heterozygosity

In polecat, the observed heterozygosity levels averaged 0.057 (*sd* = 0.133). Only three loci (ADA, MDH1 and PNP) contributed to 70.3% of the total heterozygosity. Unsurprisingly, most of scarcest alleles were especially present in heterozygotes. The observed heterozygosity levels did not significantly differ between males $\overline{H}$ = 0.055 and females $\overline{H}$=0.061 (Welch's approximate t test = 0.15 df 59 p>0.05). This level of observed heterozygosity was lower than nonbiased expected heterozygosity which reached H_E = 0.071. Although the species was uniformly widespread, polecats live a rather solitary life and it was difficult to precise the population structure. The Fis index averaged 0.198 revealing a slight deficiency in heterozygotes. This is likely to be caused by population subdivision (Wahlund's effect) as the samples were from a large area. In Western France, polecat population does not form a panmictic unit but seems to be fragmented into more or less isolated subunits. However, heterozygotes were clearly predominant in populations with 87.8% (n = 43) of individuals exhibiting at least one polymorphic locus. Only two individuals (4.1%) were heterozygous for four loci, five individuals (10.2%) for three loci and fourteen (28.6%) for two loci, heterozygotes for two or more loci totalling 42.9% of populations.

Summarising heterozygosity for 138 mammalian species, WOOTEN and SMITH (1985) reported a mean heterozygosity of 0.039 and MEROLA (1994) only noted a mean heterozygosity level of 0.022 in carnivores. Consequently, considerable allelic variation was

Table 1. *Genotypic and allele frequencies at eight polymorphic loci in polecat Mustela putorius populations from Western France.*

Loci	*n* =	SS	SF	FF	Deficiency of heterozygotes Fis (wc)	Frequency of S *p* #
ADA	*31*	0.161	0.516	0.323	- 0.043	0.419
EST-2	*49*	0.020	0.122	0.857	0.193	0.082
G6PDH	*30*	0.900	0.067	0.033	0.477	0.933
MDH-1	*49*	0.694	0.225	0.082	0.291	0.806
ME-1*	*49*	0.796	0.122	0.082	0.508*	0.857
PEP-2*	*30*	0.867	0.067	0.067	0.640*	0.900
PGM-2	*48*	0.833	0.146	0.021	0.152	0.906
PNP	*46*	0.239	0.500	0.261	0.011	0.489
mean H		0.057			0.198	
sd		*0.132*				

***** Genotype frequencies deviate significantly from Hardy Weinberg proportions P < 0.05.

detected in polecat population from Western France. Heterozygosity depletion was revealed in endangered species as in the black-footed ferret or in the cheetah *Acinonyx jubatus* (O'BRIEN et al. 1989; O'BRIEN 1994a). But the significance of heterozygote deficit was still debated (MEROLA 1994; O'BRIEN 1994b) considering that carn-ivores might be genetically less variable (MEROLA 1994). Nonetheless polecat populations from West-ern France clearly showed a level of genetic vari-ability which is higher than normally found in carnivores.

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