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ORIGINAL ARTICLE

Can low densities of carnivores result in genetic depletion? An investigation within French polecat populations

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Summary

Carnivores as top predators are commonly found in relatively low densities even in optimal habitats. Despite a lack of empirical studies, it could be suspected that long-term low density could result in genetic depletion. The genetic structure of European polecat *Mustela putorius* natural populations was investigated by allozyme electrophoresis in five distinct areas. Density estimates significantly differed among sites from 0.17 to 0.83 individuals/km^2 with an average of 0.56 individuals per km^2, resulting in a scattered distribution. Genetic structure varied among distinct populations both in number of polymorphic loci and heterozygosity. Polecats from Brittany revealed a very low observed heterozygosity ($H_O = 0.028$) whereas mean heterozygosity reached $H_O = 0.072$ in Brière. That the lowest heterozygosity levels and highest inbreeding coefficient F_{IS} were significantly associated with the lowest densities suggests that low densities may affect populations of carnivores. Both the loss of polymorphic loci and the reduction in heterozygosity rates suggest a density-dependent effect and population density can be arguably regarded as a factor affecting genetic diversity in top carnivores.

Introduction

Decline in genetic variation can be a critical question for species conservation in a changing environment. It might be predicted that levels of genetic diversity should decrease with the population size (Soulé 1976; Amos & Balmford 2001), reducing the adaptive potential of the species (Frankham 1995, 1996; Frankham *et al.* 2002). Reduced genetic diversity within populations is a function of population size but drastic population decrease entails a bottleneck effect inducing a loss of genetic variation. Restricted population size could also result in a decline of fitness due to decreased heterozygosity and in turn strengthened the population decline (Reed & Frankham 2003). In the case of isolated populations, this loss can be irreversible, migrations no longer contributing to correct the variability (Ellstrand & Elam 1993). Influence of population size on genetic variation was well documented (see Frankham 1996) but that low density could induce a local depletion in genetic diversity may also be suspected. Density measures do not only indirectly estimate the size of a population but also focus on the dispersion of individuals. Panmixia could be seriously altered in a sparsely populated area and it might be predicted that same-sized populations with disparate densities may exhibit very different genetic diversity.

Carnivores showed intrinsically small effective number because as top predators they are found in relatively low densities even in optimal conditions. Due to natural process or human induced, rarity constitutes one of the major patterns of species vulnerability (Meffe & Carroll 1997). Numerous mustelid

carnivores, such as River otter or European mink, for instance, suffered important decline in their populations because of trapping or toxic contamination (Macdonald & Mason 1994; Kruuk 1995; Lodé *et al.* 2001). The European polecat *Mustela putorius* is still a widespread carnivore in the Western Palearctic but considered as being of unfavourable conservation status and probably endangered (Anonymous 1995; Birks 1998). This opportunistic mustelid occupies a great variety of habitats exploiting mainly anuran and rodent prey (Lodé 1994, 1997). Nevertheless, the species did not exhibit an uniform distribution and the range was fragmented into more or less separated population units. Polecat populations present variations in abundance within their range mainly linked to the habitat quality (Blandford 1987). Furthermore, most of mustelids suffered from systematic persecution in Western Europe as they were regarded as hunters' competitors for small game and long-term trapping has been proved to have locally a relevant effect on mustelid population (Langley & Yalden 1977). Based on a territorial spacing pattern as in many carnivores, polecat exhibited a polygynous mating system (Lodé 2001) but males showed a very strong intolerance towards congeners resulting in scattered populations (Lodé 1996; Lodé *et al.* 2003).

Polecat populations could show various and locally low densities because they are trapped as pests in many areas. Consequently, it could be expected that the genetic diversity of polecat populations could differ among areas and it could be suspected that long-term low density could result in genetic depletion.

In this paper, the genetic variability within and among European polecat natural populations from western France was investigated using allozyme electrophoresis and population densities were estimated. This study aims at assessing whether: (i) genetic variability would differ among populations; and (ii) the variability should be related to the density of populations supporting the prediction that low density may result in loss in genetic variation. If gene flow were altered among units, heterozygosity within units would decrease because of random drift. But, besides the Wahlund effect (Wahlund 1928) the whole population variability would increase because different alleles are fixed in different populations (Wright 1978; Ridley 1996; Lodé 1998). Estimating loss of genetic variability and heterozygosity in polecat populations might indicate how natural population units react to an increase in the dispersion of individuals because of a low density and might provide valuable information for effective management and biological conservation of rare carnivores.

Materials and methods

Population density

The study was carried out in five areas from western France, Brittany, Brière, Anjou, Vendée and Aquitaine during 1994–96 (Figure 1). Mean temperature in January 1989, respectively, reached 2.9, 3.7 and 0.7°C in Pays de Loire, Brittany and Aquitaine, and 27.1, 25.7 and 28.9°C in July 1989. In January 1995, temperatures averaged 2.9, 3.3 and 3.0°C, and in July 27.6, 25.0 and 29.3°C. Precipitation equally distributed during the year averaged 700, 800 and 600 mm, respectively, for Anjou, Brittany and Aquitaine with <20 frozen - days/year. In each area, live trapping sessions were conducted in two distinct sites for each period between November and February, except in Aquitaine 1994 where trapping was only made in one site. The traps were set in the apparently most favourable habitats for polecat for each site along hedges and watercourses (Lodé 1994, 2001). Individuals were live-trapped using wood box traps and marked with electronic microchips. Because

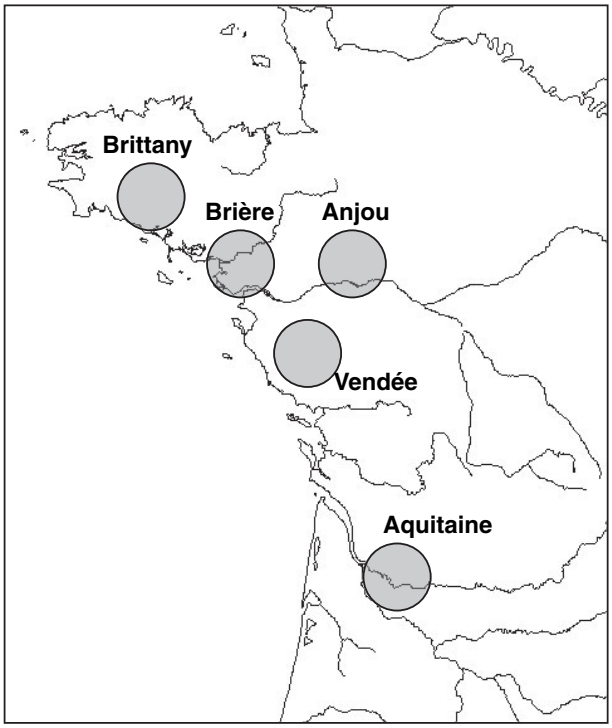

Figure 1 Locations of the studied natural population units of *Mustela putorius* in France.

the sexual maturity is reached approximately at 9–11 months and birth occurred in May to June, all caught individuals were adult. Polecats were sexed, weighed, marked and released and the index of polecat numbers was assessed as $I_{TN} = n$ individuals/n trap nights × 1000 respecting a total of 4200 trap nights per period. Twenty traps were set by 300-m intervals and draining an approximate surface of 6.5 km^2 following the estimate of Thompson (1994) arbitrary determined as 500-m wide zone around the outermost traps. Estimate of density D_m was made directly by reporting the number of captures to this arbitrarily estimated surface (Thompson 1994; Jedrzejewski *et al.* 1995).

Nevertheless, to take the amplitude of real movements and the difference between sexes into account, another density estimation D_{cor} has been calculated by applying a correction in surface measures (cf. Mills 1996). This surface calculation is corrected by the dispersion variance of animals as it has been assessed by the radiotracking of 23 individuals (Lodé 1994, 2001). The dispersion variance is estimated by bringing the greatest monthly distance between two locations to the average of observed monthly distances and corresponds to an average surface of 398-m diameter for males (monthly home range 1.184 km^2) and 289 m for females (monthly home range 0.441 km^2). Consequently, the estimation of the corrected surface for males reached a total of 5.17 km^2 for Aquitaine (one site) and 10.34 km^2 for other areas, and 3.63 km^2 (Aquitaine) and 7.26 km^2 (two sites) for females. Although this estimation may provide a good approximation of the density in studied propitious habitats, it is especially its relative value that is considered here. The method having been standardized on every site provided comparable information among areas and the density is averaged from the two trapping sites for each area. Changes in trapping success between the two considered periods were tested by the chi-squared test. Differences between the different areas were tested using the Kruskall–Wallis test with Tukey–Kramer multiple comparison tests.

Sample collection and electrophoresis

A total of 72 road-killed polecats was opportunistically collected from the five distinct areas, Brittany, Brière, Pays de Loire and Aquitaine between 1994 and 1997 and immediately frozen (authorization DPN 95–97). From muscle tissue stored at −70°C, crude protein extract was used for electrophoresis. An equal volume of distilled water containing buffer was added and tissue samples were centrifuged at 10 000 ***g***. Electrophoresis of soluble proteins was carried out in horizontal starch gel using two continuous buffer systems Tris–citrate pH 6 and Tris–EDTA borate pH 8. Thirty-nine presumptive loci encoding 25 enzyme systems were scored following Pasteur *et al.* (1987); Murphy *et al.* (1990) and Rothe (1994) (Table 1) and alleles were designated alphabetically in decreasing order of mobility relative to the most anodal one. Loci successfully resolved were *AAT-1* and *AAT-2* (EC 2.6.1.1), *ACO-1* and *ACO-2* (EC 4.2.1.3), *ADA* (EC 3.5.4.4), *AK* (EC 2.7.4.3), *CK-1* and *CK-2* (EC 2.7.3.2), *DDH-1* and *DDH-2* (EC 1.8.1.4), *EST-1* and *EST-2* (EC 3.1.1.1), *FBP* (EC 3.1.13.11.), *FUMH* (EC 4.2.1.2), *GLY2DH* (EC 1.1.1.29), *G6PDH* (EC 1.1.1.49), *GPI* (EC 5.3.1.9), *HK-1*, *HK-2* and *HK-3* (EC 2.7.1.1), *IDH-1* and *IDH-2* (EC 1.1.1.42), *LDH-1* and *LDH-2* (EC 1.1.1.27),

Table 1 Allelic variations for polymorphic loci in five polecat populations from western France and levels of observed and non-biased expected heterozygosities

	Brittany		Anjou		Brière		Vendée		Aquitaine	
Loci	*p*	*q*	*p*	*q*	*p*	*q*	*p*	*q*	*p*	*q*
ADA	0.156	0.844	0.45	0.55	0.625	0.375	0.727	0.273	0.817	0.193
EST-2	0.143	0.857	0.03	0.097	0.208	0.792	0.05	0.95	0.346	0.654
G6PDH	1		0.885	0.115	1		0.773	0.227	0.778	0.222
MDH-1	0.844	0.156	0.7	0.3	0.875	0.125	0.864	0.136	0.769	0.231
ME	0.937	0.063	0.825	0.175	0.708	0.292	0.818	0.182	0.461	0.539
PNP	0.625	0.375	0.425	0.575	0.583	0.417	0.409	0.591	0.269	0.731
PEP-LA	0.833	0.167	0.823	0.107	1		0.954	0.046	1	
PGM-2	0.844	0.156	0.875	0.125	0.864	0.136	0.773	0.227	0.961	0.039
SDH	0.900	0.200	0.559	0.441	0.800	0.200	0.227	0.773	0.269	0.731
H_O	0.0281		0.0631		0.0721		0.0677		0.0511	
H_E	0.0551		0.0751		0.0807		0.0895		0.0754	
n	16		20		12		11		13	

MDH-1 and *MDH-2* (EC 1.1.1.37), *ME-1* and *ME-2* (EC 1.1.1.40), *MPI* (EC 5.3.1.8), *PEP-1* and *PEP-2* (EC 3.4.11.1), *PGDH* (EC 1.1.1.44), *PGM-2* (EC 2.7.5.1), *PNP* (EC 2.4.2.1), *SDH* (EC 1.1.1.14), *SOD* (EC 1.15.1.1), *TPI* (EC 5.3.1.1) and two non-specific proteins.

F-statistics were calculated using GENETIX software (Belkhir *et al.* 2000) for genetic frequencies, observed H_O and non-biased expected heterozygosity H_{NB}. The distribution of genetic variation among populations was estimated using Wright's variance $G_{ST} = 1 - H_s/H_t$ where H_s and H_t were heterozygosity within populations and total heterozygosity when all individuals were pooled. Following GENETIX procedure, the genetic distance $D = -\ln(1 - G_{st})$ between population pair (Nei 1972; Reynolds *et al.* 1983) was calculated. One-way analysis of variance with Bonferroni multiple comparisons test was used to appreciate variations in individual heterozygosities. To estimate the possible divergence among areas, genetic distances were assessed based on a NEIGHBORNET network generated by the SPLITSTREE (http://www-ab.informatik.uni-tuebingen.de/software/splits/welcome_en.html).

Results

Population density

A total of 46 individuals (25 males and 21 females) were captured on different areas. The number of captures did not significantly differ between the first and the second period ($\chi^2 = 0.22$, NS).

The trap night index averaged $I_{TN} = 4.31$ SD = 2.019 ($I_{TN} = 4.524$ in 1994/95 and $I_{TN} = 3.809$ in 1995/96) but significantly varied (KW = 6.53, $p < 0.01$) among sites, excepted between Anjou region and Brière (Tukey–Kramer test $P < 0.05$). Density averaged $d_m = 0.398$ individual/km^2 (SD = 0.186) and corrected density averaged $d_{cor} = 0.561$ (SD = 0.266).

At each period, densities significantly varied among areas (KW = 6.24, $p < 0.01$ and KW = 6.17, $p < 0.035$ respectively) except between Brière and Anjou where polecat numbers remained stable. The highest value was reached in Brière ($d_m = 0.577$, $d_{cor} = 0.828$) and the lowest in Brittany ($d_m = 0.116$, $d_{cor} = 0.166$). Density measures were strongly correlated ($R_{Spearman} = 1$, $p < 0.02$).

Genetic structure

Nine of the 39 presumptive gene loci were found polymorphs at $p = 0.05$ in the total sample corresponding to 23.1% of polymorphic loci with an effective number of 1.23 alleles per locus. The mean heterozygosity (H_O) reached 0.0513 (SD = 0.1141, Table 1). The number of polymorphic alleles differed among studied populations. Populations from Brittany and Aquitaine showed the smallest level with eight (20.5%) and seven polymorphic loci (17.9%), respectively, whereas the polymorphism reached 25.8% in Vendée.

The observed heterozygosity significantly differed among population units ($F = 8.154$, d.f. = 3.53, $p < 0.001$, Bartlett's test for homogeneity = 1.32, NS) stressing that variations among population units was significantly greater than within population units. The mean rate of observed heterozygosity (H_O) varied according to the considered population unit, decreasing in Brittany to 0.028 while it reached its highest level in Brière with 0.072 ($p < 0.05$ Bonferroni multiple comparisons test). Thus, differences in mean heterozygosity were clearly associated with the assessed densities. Because population samples with similar sizes showed both the highest and the lowest level of heterozygosity, these results could not be considered as sample size dependent. The different populations were in Hardy–Weinberg equilibrium except for PNP in Brittany ($G = 4.68$, $p < 0.05$) and ME in Pays de Loire ($G = 5.66$, $p < 0.02$). Population from Brittany revealed the most important heterozygote deficit as shown by the inbreeding index F_{IS} (Table 2). Although only five data were available, estimates of densities were both related to levels of heterozygosities (Spearman correlation for small samples $r = 0.8$, $p < 0.019$) and to inbreeding index ($r = -1.0$, $p < 0.016$) revealing a high genetic depletion related to densities (Figure 2).

Populations clearly differed and multiloci G_{ST} averaged 0.1378 (Table 3). Unsurprisingly, genetic distances among population units increased from north to south with geographical distances and were the strongest between Brittany and Aquitaine (Figure 3).

Table 2 Density assessment versus genetic F_{IS} in five polecat populations from western France (index of inbreeding F_{IS} was averaged for multiple loci)

	F_{IS}	Mean d_{cor}	Mean d_m	ITN
Brittany	0.498	0.166	0.116	1.25
Brière	0.112	0.828	0.577	6.25
Anjou	0.165	0.731	0.501	5.42
Vendée	0.252	0.654	0.489	5.31
Aquitaine	0.333	0.428	0.308	3.33

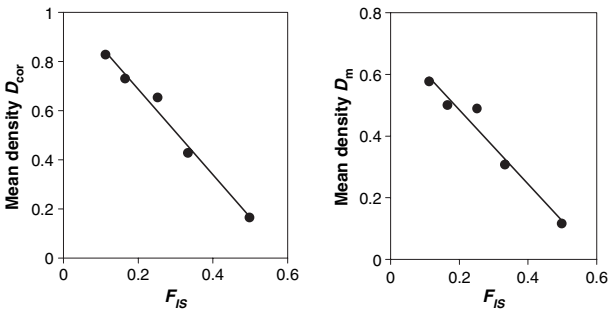

Figure 2 Relation between populations density and inbreeding coefficient F_{IS} in population units of *Mustela putorius* in France.

Table 3 Genetic distance *D* among five polecat populations (following GENETIX procedure)

D	Brière	Anjou	Vendée	Aquitaine
Brittany	0.0767	0.0771	0.2765	0.3804
Brière		0.0309	0.1044	0.1358
Anjou			0.0383	0.1219
Vendée				0.0413

Discussion

The prediction that a small size population should have less genetic variation has a considerable theoretical support (Nei *et al.* 1975; Soulé 1976; Leberg 1992; Frankham 1995). Severe decline in number through a bottleneck should entail a drastic loss of variability (Nei *et al.* 1975; O'Brien 1994). But in polecat, low density was associated with the lowest genetic variability; that long-term low density could result in genetic depletion could be predicted in endangered species. Thus, otter populations are presumed to exhibit low genetic variability as a result of long-term low densities and decline (Effenberger & Suchentrunk 1999).

For instance, polecat populations with 23% of polymorphic loci were characterized by a variability comparable with that recorded in American marten (33.3% Mitton & Raphael 1990), in sea otter (19.4% Lidicker & Maccollum 1997) or in grey wolf (18.9% Kennedy *et al.* 1991; 10% Randi *et al.* 1993). Predictably, the loss of genetic variation in polecat population from Brittany was directly linked to a lower number of polymorphic loci following Nei's hypothesis that a bottleneck affects more allelic diversity than heterozygosity (Nei *et al.* 1975; Leberg 1992). Although every polecat population showed a heterozygote deficit, the deficiency was very high in polecat from Brittany revealing a high inbreeding index. In Brittany, polecats mainly frequented oligotrophic brooks and it could be presumed that resource dispersal affects density. By contrast, the polecats from Brière largely exploited a marshy area very productive for trophic supply (Lodé 1994). Finally, in Aquitaine or Pays de Loire, the mustelid occupied chiefly eutrophic habitats with ponds and slow streams (Lodé 1994).

Unsurprisingly, genetic distance increased with geographical distance between populations. Inbred populations commonly appear distant from other populations when genetic distances were based on alleles frequencies, so that this does not allow any conclusion about divergence times and phylogeny. Because only 14% ($G_{ST} = 0.138$) of the gene diversity was between populations while 86% was within population units, the loss of genetic diversity may be partly attributed to restricted gene flow. Although no patent biogeographical obstacle enables to account for such a difference, genetic diversity in polecats was found to be affected by distance. In addition, the small size of the population could restrain the number of emigrants (Slatkin 1985) preventing most females from finding mates. Actually, following the Allee effect hypothesis (Allee *et al.* 1950) predicting that reproduction rates should be altered in poor habitats with extensive scattered population, heterozygote deficit may result from low densities. Mustelid populations seemed to be structured in very small reproduction units distributed at irregular intervals in the more favourable habitats (Lodé 2001). The strong individualistic habits of the species can also restrict breeding exchanges (Lodé 1996; Lodé *et al.* 2003) but heterozygote deficiencies should also be attributed to the sexual system of polygynous species (Bonnell & Selander 1974). Although polecats

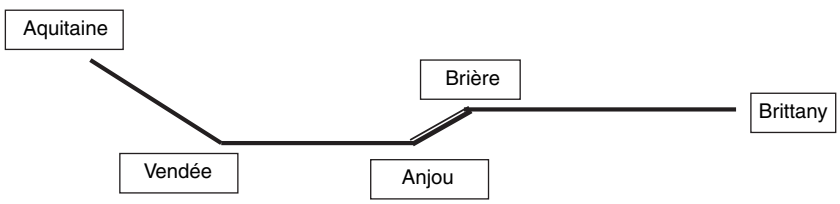

Figure 3 Graph representation of genetic distance among five polecat *Mustela putorius* populations from western France.

showed a polygynous mating structure, differences in heterozygosities were, however, evidenced among polecats from distinct areas and this pattern of heterozygosity loss did not only result from their sexual system.

Carnivores such as otters or polecats are both at the top of the food chain and required precise habitat qualities but, as top predators, they are always found in low densities even in optimal habitats and basically exhibited a real vulnerability to demographic depletion. Most of the top carnivores are endangered species showing declining populations (Meffe & Carroll 1997). Given the importance of genetic diversity for short-term fitness, carnivores are especially vulnerable when reproduction rates were altered. The heterogeneous distribution and the low density of polecat populations clearly constituted serious constraints, which could affect maintenance of genetic diversity. So, whatever the density estimate, the heterozygosity rates remained the weakest when densities were the lowest. Bottleneck generally induced rather a reduction of locus polymorphism than a decrease of heterozygosity (Charkraborty & Nei 1977; Leberg 1992). A loss of alleles exists at some phases of the density cycle in snowshoe hares (Lidicker *et al.* 2000) but in polecat populations from Brittany, the heterozygote deficiency did not only proceed from the loss of rare alleles so that it could be suggested that the decline in density induced a real heterozygote depletion. The decrease in heterozygosity did not only result from the reduction of effective number but also from the scattered distribution of individuals in space, i.e. the density, preventing individuals to find mate partners. In European mink, hybridization events increased with the reduction of available mating partners (Lodé *et al.* 2005). This density-dependent effect could be regarded as a variation of the neighbourhood effect (Wright 1946).

Thus, decreasing density drastically reduced genetic diversity affecting the number of polymorphic loci and lessening the heterozygosity within populations. Long-term low densities operate as rarity making species outstandingly vulnerable to genetic depletion. The problem of low density expands the problem of the population size for an effective conservation and, threatening the opportunity for mates, low density may be arguably thought as an important factor for the genetic conservation of natural populations.

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