Genetic divergence without spatial isolation in polecat *Mustela putorius* populations

T. LODÉ

Laboratoire d'Ecologie Animale, Faculté des Sciences, Université d'Angers Belle-Beille, Angers, France

Keywords:

endogamy;
genetic divergence;
habitat preferences;
Mustela;
polymorphism;
sympatric differentiation.

Abstract

Understanding how genetic divergence could exist without spatial isolation is a fundamental issue in biology. Although carnivores have previously been considered as having a weak genetic variability, polecats *Mustela putorius* from eight distinct populations exhibited both a strong polymorphism (17.5–22.5%) and a substantial allele effective number reaching $N_e = 1.12$. Heterozygosity ranging from $H_o = 0.031$–0.063 significantly differed among populations, while the mean F_{IS} averaging 0.388 stressed a real deficiency of heterozygotes. Observed heterozygosity levels among populations did not correlate with any habitat types but were clearly associated with habitat diversity index. The habitat structure in polecat home range corresponded to habitat mosaic structure in which discrete habitat types alternated causing multifactorial constraints that may favour heterozygosity. Allozymic frequencies within populations did not vary with dominant habitat. But in the *Tyrosinase-1*, the rare homozygote BB, resulting in a 'dark' phenotype, was found much more in deciduous woods than the homozygote AA showing the 'typical' pattern. Thus, the genetic basis for a character differentiation was here evidenced in a remarkable situation without spatial isolation. Further, the very low proportion of heterozygotes for this locus suggests a disruptive effect and supports the prediction of intermediate phenotypes being at a disadvantage. This heterozygote deficit may also result from an assortative mating intra phenotype (*homogamy*). The divergence in polecat phenotypes showed that genetic differentiation can be induced by subtle variations in environment, a situation that is likely to be frequent in most natural populations, and emphasized the adaptive nature of habitat preference.

Introduction

How habitat preference leads to divergence and discrete polymorphism within or among populations is a fundamental issue in evolutionary ecology. Nonetheless, genetically based differentiation of phenotypes has rarely been proven to be adaptive in animals (Garland & Adolph, 1991; Endler & Théry, 1996; Blondel *et al.*, 1999). Although intraspecific variability is the rough matter of evolution, population divergence requires breeding isolation. According to Mayr's allopatric theory (1963), restriction in gene flow often results from geographical barriers or long distance separating populations.

Nevertheless, numerous nonisolated populations can display a wide polymorphism with distinct morphs coexisting in sympatry. In natural populations, some variations were so subtle that their adaptive significance may be debated (Abrams, 1990; Robinson & Wilson, 1994). But from discrete phenotypic differences, there is growing evidence of a trend towards population differentiation regarded as potential sympatric speciation (Rice & Hostert, 1993). For instance, differentiated morphs could subsist in some bluegill and pumpkinseed sunfish, brown trout, arctic and brook charr and African cichlid

Correspondence: T. Lodé, Laboratoire d'Ecologie Animale, Faculté des Sciences, Université d'Angers Belle-Beille, F-49045 Angers, France.
e-mail: thierry.lode@univ-angers.fr

populations (Ferguson & Taggart, 1991; Skulason *et al*., 1993; Robinson & Wilson, 1994, 1996a; Robinson *et al*., 1993; Malmquist *et al*., 1992; Schliewen *et al*., 1994). Other examples are mentioned in amphibians and birds (Pfenning, 1990; Smith, 1993; Butlin & Tregenza, 1997) but such a divergence is not obvious in mammals. Further, the importance of genetic polymorphism is still poorly known in mammals (Wooten & Smith, 1985; Hartl *et al*., 1988; Merola, 1994).

The evolutionary concern arises because distinct morphs have identical life history in contrast to allopatric populations. Such a differentiation should result from both intraspecific competition and discrete variation in resource, i.e. a resource polymorphism (Skulason & Smith, 1995; Tregenza & Butlin, 1999). Whatever the model of population genetics, ecological pressures should disadvantage intermediate phenotypes inducing disruptive selection (Dieckman & Doebeli, 1999; Kondrashov & Kondrashov, 1999). Competition is regarded as a diversifying force occasioning character differentiation. Although character differentiation is related to differences in resources in species showing strong competition, heritability and genetic basis of this phenomenon are rarely evidenced (Grant, 1975; Abrams, 1990).

As a result of strong intraspecific competitive interactions, European polecats *Mustela putorius* mostly live a solitary life with an area restricted search predation pattern (Lodé, 1996, 2000). Polecats display a polyphenotypism regarding their coat colour and their size, traits often associated in mustelid genome (Lynch & Hayden, 1995). In addition to the 'typical' morphotype, a 'dark' phenotype is noticed, based on the body mass, the fur pigmentation and the facial pattern. Polecats show ecological plasticity exhibiting a great variety of feeding tactics and inhabiting various habitats such as marsh, farmland, steppe and deciduous forest (Blandford, 1987; Lodé, 1994, 1997). Genetic basis for habitat selection is poorly documented but fitness is assumed to be enhanced in preferred habitat (Jaenicke & Holt, 1991; Martin, 1998). While habitat features may influence gene flow and allopatric divergence among populations on a regional scale, it could be hypothesized that microhabitat choice may result in different fitness, thus contributing to sympatric divergence. Because of their genetic variability (Lodé, 1998), their use of diverse habitats, their feeding tactics and the importance of intraspecific competition, polecat populations constitute an interesting model for examining the evolutionary significance of genetic divergence.

I investigated the genetic variability in eight distinct polecat populations to address two evolutionary questions in the general scope of the sympatric speciation debate: firstly, what is the degree of genetic differentiation among populations considering allelic variation and heterozygosity; secondly, could genetic and phenotypic divergence within populations be related to habitat preferences. The electrophoretic analysis of allozymes is a very relevant method for studying multilocus genetic divergence in natural populations (Ayala, 1976; Ferguson & Taggart, 1991) and a great deal of attention was paid to the examination of Tyrosinase, one of the enzymes involved in fur colour expression.

Materials and methods

Data collection

Polecats *M. putorius* were sampled between 1996 and 1999 from eight populations in France, Brittany (mean temperature of July: 17.5 °C, mean temperature of January: 5.8 °C, annual precipitation 880 mm), Brière (18.5 °C, 5.0 °C, 850 mm), Anjou (20 °C, 5 °C, 650 mm), Sologne (19.4 °C, 0.4 °C, 600 mm), Morvan (21 °C, 0.0 °C, 1050 mm), Vendée (18.2 °C, 6.0 °C, 750 mm), Limousin (20.1 °C, 1.3 °C, 900 mm), and Aquitaine (22.0 °C, 6.6 °C, 850 mm) (Fig. 1). Polecats are mainly found in wetlands, ponds and streams, but also in oligotrophic forest brooks in Brittany, Morvan and less frequently in Limousin. Habitats in Brière, Anjou, and Vendée are mostly eutrophic marsh ditches, ponds or slow streams. Polecats are regarded as pests and extensively trapped. I obtained muscle tissue samples from trapped or road-killed animals from trappers, taxidermists and naturalist associations (DPN authorization no. 98/717/AUT).

Habitat selection

Only clearly localized adult animals which were successfully investigated by starch gel electrophoresis have been retained ($n = 114$). Each polecat was weighed and sexed and detailed habitat features were described in an area of 1 km^2 around the point of discovery (radius = 564 m). A surface of 1 km^2 corresponds to the mean home range size recorded in western France from radiotracked polecats (Lodé, 1994) while daily movements reached 600 m on average (Blandford, 1987; Lodé, 1993). Measurements were taken on variables related to vegetation structure. The cover rate of each vegetation type was used as six descriptive habitats:

1 deciduous woods, percentage of surface covered by woods mainly composed of Oaks *Quercus* sp., Ashes *Fraxinus excelsior* and elms *Ulmus minor;*

2 coniferous or mixed forest;

3 willow groves composed of *Salix* sp. and *Populus nigra* and marshes, including peat-bogs and reed-beds;

4 natural meadows, with herbaceous cover often hedged by oaks and ashes;

5 cultivated fields; and

6 urban and peri-urban zones. Variables were measured on 1 : 25000 topographic maps.

Habitat diversity was calculated from habitat frequencies using Levin's index (1968) $B = 1/\sum Pi^2$ where Pi is the cover rate of each habitat type. Statistical tests

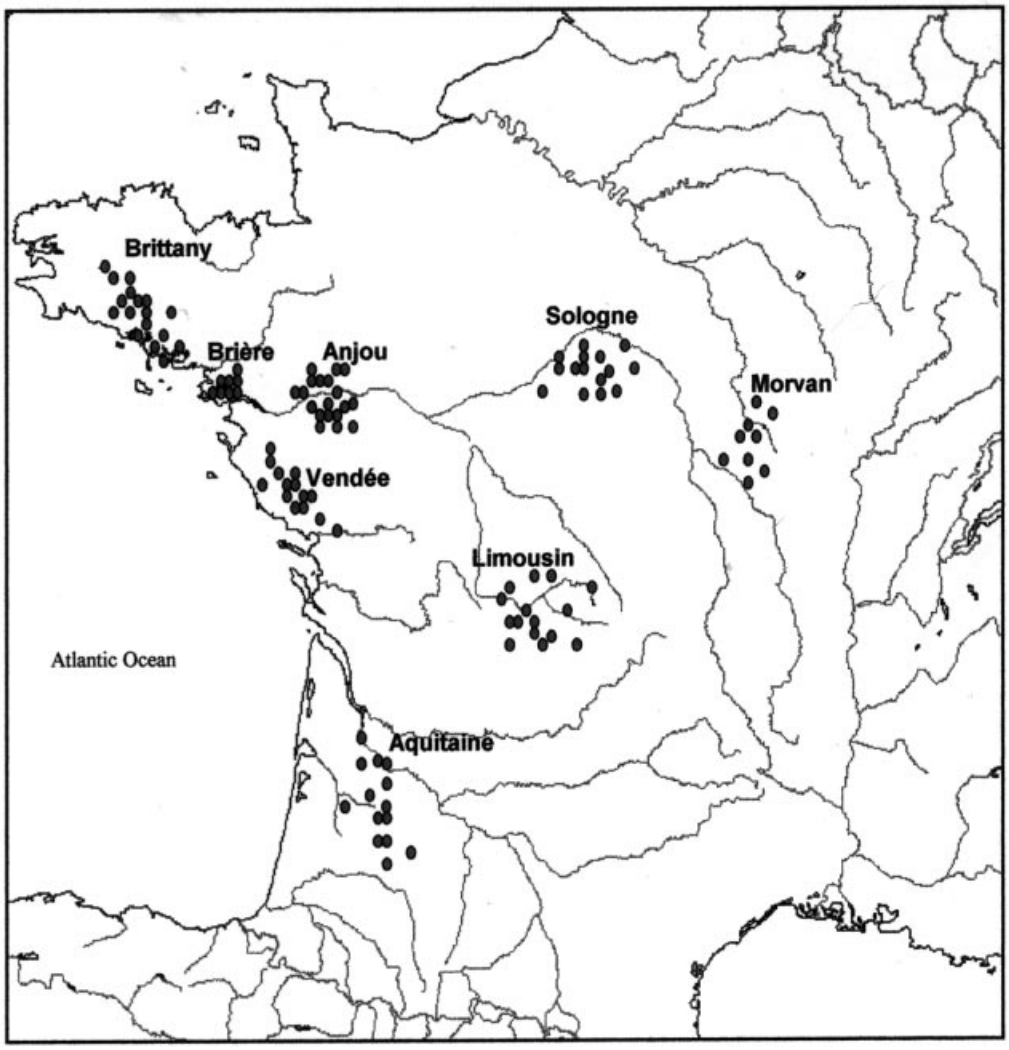

Fig. 1 Location of polecat *M. putorius* samples in eight populations from France (dots are individuals sampled).

(one-way ANOVA or Kruskal–Wallis) and correlation were performed using Pcsm program considering:
1 variations among populations (pooling data from individuals for each population); and
2 differences among genotypes (pooling all data).

Electrophoresis analysis

Tissue samples removed from each individual were immediately frozen and stored until electrophoresis was performed. Crude protein extracts from this tissue, macerated in equal volume of distilled water were centrifuged at 10 000 g for 15 min at 4°. Electrophoresis of soluble proteins was carried out in starch gel (Sigma) using three buffer systems, Tris-citrate pH 6, Tris-citrate pH 8 and Tris-Edta-borate pH 8 (see Lodé, 1998). Slices were stained for 27 enzymes encoded by 40 gene structure loci following Pasteur *et al*. (1987), Murphy *et al*. (1990) and Rothe (1994) procedures. 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), *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), *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), *TYR1* and *TYR2* (EC 1.14.18.1) and two nonspecific proteins. Allozymes were scored alphabetically according to the mobility of their products.

Tyrosinase converts Tyrosine to Dopachrome which leads to dark eumelanin but the pigmentation pattern is also under the control of several other loci such as *TRP1*, *TRP2*, or *MC1-R* (Searle, 1968; Willis, 1989). Although fur pattern in polecat varied from a clear coat in winter to a darker one in summer, the polymorphism in the Tyrosinase locus resulted in three identified coat colour phenotypes in the wild, the 'typical' one with a distinct bandit mask, a 'dark' phenotype with no mask apparent and dark fur and one intermediate (heterozygote) with only two light spots above eyes. Another recessive allele determined albinistic phenotype or Tyrosinase positive albinos mainly found in the domestic ferret. Further, some erythristic polecats in which dark guard hairs were replaced by red ones were found in Britain (Blandford, 1987; Birks & Kitchener, 1999), a phenotype probably derived from another allelic variant of Tyrosinase producing phaeomelanin.

Genotypic frequencies at each locus were tested for fit to Hardy–Weinberg equilibrium and F-statistics were calculated using Genetix (Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. 1996–98, Genetix version 3.3, Laboratoire Génome & Populations, CNRS UPR 9060, Montpellier, France) and Popgen32 software (Yeh, F., Yang, R.C., Boyle, B.J., Ye, Z.H. & Mao, J.X. 1997, Pop Gen 3.2. Mol Biol Biotech Centre, Univ Alberta). The effective number of alleles was assessed as in Kimura & Crow (1964) revised by Nei (1987). Observed (H_o) *and* expected (H_E) average heterozygosities (Nei, 1978) were estimated and heterozygote deficiency was based on the F_{IS} index as in Wright (1978).

Results

Differentiation among populations

Out of 40 successfully scored loci, 10 (25%) were found polymorphic and polymorphism ranged 17.5–22.5% in populations. The mean effective number of alleles reached 1.12 with no significant difference among populations ($F = 0.1$, d.f. $= 7$, $P > 0.05$). Percentage of polymorphic loci was not associated with sample size ($r_S = 0.155$, $P = 0.7$).

Allozymic frequencies varied among polecat populations and mean observed heterozygosity, ranging from $H_O = 0.031$ in Brittany to $H_O = 0.063$ in Anjou, significantly differed among populations ($F_{7,113} = 2.15$, $P < 0.05$) (Table 1). Mean fixation value averaging $F_{IS} = 0.388$ indicated a deficiency of heterozygotes. Most of the gene frequencies of polymorphic locus (*ADA*, *EST-2*, *MDH-1*, *NP*, *PEP-2*, *SDH*) were in agreement with Hardy–Weinberg equilibrium in at least six populations. Nevertheless, significant deviations from HW would imply large deficiencies to be manifest. In fact, only *ME-1*, *PGM-2* and especially *TYR-1* showed a significant deviation from Hardy–Weinberg as a deficiency of heterozygotes in four populations and even up to seven populations in the case of *TYR-1* (Table 1).

Habitat traits in estimated polecat home ranges showed relatively few differences among populations (Kruskal–Wallis test, KW $= 1.8$, $P > 0.05$). The home range of polecats mainly included deciduous woods (mean 31.3%) and willow groves and marshes (mean 29.7%) while cultures (mean 11.7%) and mixed woods (mean 4.2%) were almost neglected (Table 2).

The levels of heterozygosity of eight polecat populations were correlated with habitat diversity index ($r = 0.953$, 90.9% of explained variance, $F = 59.9$, $P < 0.001$) (Fig. 2). Unsurprisingly, the F_{IS} index was also negatively correlated with habitat diversity index ($r = -0.863$, 74.4% of explained variance $F = 17.4$, $P < 0.01$).

The multiple regression analysis between genetic parameters and other habitat types of polecat populations showed that habitat type did not significantly correlate with the effective number of alleles ($r_{mult} = 0.899$, 80.76% of explained variance, $F = 0.7$, $P > 0.05$, Durbin–Watson $= 1.337$) (see web materials). None of the habitat types were significantly associated with either the observed heterozygosity levels in distinct populations ($r_{mult} = 0.956$, 91.4% of explained variance, $F = 1.77$, $P > 0.05$, Durbin–Watson $= 1.34$) or the F_{IS} index ($r_{mult} = 0.975$, 95.3% of explained variance, $F = 3.35$, $P > 0.05$, Durbin–Watson $= 1.34$).

Habitat preference and differences among genotypes

For 37.7% of polecats ($n = 43$), the estimated home range included more than 50% of willow groves and marshes while the home range was dominated (more than 50%) by deciduous woods for 42 polecats (36.8%). For 29 polecats, the home range consisted of more than 50% of natural meadows. No polecat home range included more than 30% of cultures, mixed woods or urban zones.

Observed heterozygosity levels did not significantly differ among polecats inhabiting willow groves and marshes ($H_O = 0.051$, $SD = 0.031$), deciduous woods ($H_O = 0.043$, $SD = 0.028$) or natural meadows ($H_O = 0.045$, $SD = 0.032$) ($F_{2,113} = 0.73$, $P > 0.05$). Nevertheless, although not statistically tested, the heterozygote deficiency as revealed by the F_{IS} index was higher in deciduous woods (0.514) than in meadow (0.418) or marshes (0.351).

The uncommon allele C in *MDH-1* was evidenced everywhere while the rare allele B in *NP* was only found in polecats occupying mainly deciduous woods. Whatever the locus, all other alleles were discovered in every habitat types. For most of the considered loci, gene frequencies did not differ from one habitat type to another (see web material).

Only for the *TYR-1*, gene proportions significantly differed according to the habitat type ($G_{woolf} = 12.8$,

Table 1 Observed heterozygosity, tests for Hardy–Weinberg equilibrium, mean expected heterozygosity and F_{IS} index for 10 polymorphic loci in polecat *M. putorius* populations.

	Anjou	Vendée	Brière	Brittany	Aquitaine	Morvan	Limousin	Sologne
Polymorphism at 0.05	22.5%	22.5%	20%	20%	20%	20%	17.5%	17.5%
ADA								
H_O	0.600	0.286	0.417	0.063	0.385	0.100	0.357	0.600
χ^2 =	0.69	0.54	0.49	11.6 **	0.57	7.7**	1.17	0.53
G =	0.70	0.48	0.51	7.9***	0.96	8.1***	1.16	0.54
EST-2								
H_O	0.050	0.154	0.750	0.125	0.077	0.000	0.000	0.000
χ^2 =	0	0.04	1.09	8.2***	10.0***	0	0	0
G =	0	0.08	0.92	7.3**	10.7***	0	0	0
G6PGDH								
H_O	0.077	0.071	0.000	0.000	0.222	0.125	0.333	0.000
χ^2 =	7.7**	9.9***	0.16	0	1.77	5.1*	1.17	0
G =	4.1*	7.4**	0.28	0	1.46	4.9*	1.15	0
MDH-1								
H_O	0.300	0.286	0.250	0.187	0.308	0.300	0.100	0.333
χ^2 =	1.98	0.28	0.16	4.0	0.42	0.69	7.1**	2.03
G =	1.98	0.50	0.29	3.4	0.38	0.62	7.4**	2.03
ME-1								
H_O	0.150	0.357	0.250	0.125	0.154	0.100	0.167	0.133
χ^2 =	5.6*	0.51	2.41	0.03	6.96	6.7**	6.1*	8.7***
G =	4.2*	0.88	2.43	0.07	7.54	5.9*	6.4**	9.3***
NP								
H_O	0.650	0.357	0.417	0.250	0.385	0.300	0.357	0.400
χ^2 =	1.86	1.43	0.5	2.24	0.05	0.69	0.11	0.8
G =	1.93	1.44	0.5	2.01	0.05	0.62	0.10	0.8
PEP-2								
H_O	0.071	0.071	0.000	0.187	0.000	0.000	0.000	0.000
χ^2 =	8.3***	0	0	4.0	0	0	0	25.1***
G =	4.2*	0	0	3.36	0	0	0	8.4***
PGM-2								
H_O	0.150	0.071	0.273	0.125	0.077	0.100	0.000	0.067
χ^2 =	2.69	10.6***	0.18	6.8**	0	6.7**	0	8.9***
G =	1.83	9.7***	0.32	5.2*	0	5.9*	0	4.3*
SDH								
H_O	0.412	0.357	0.200	0.062	0.385	0.200	0.385	0.267
χ^2 =	0.65	0.11	2.11	0	0.05	3.49	0.83	3.74
G =	0.65	0.10	1.66	0	0.05	3.25	0.83	3.85
TYR-1								
H_O	0.053	0.083	0.083	0.125	0.077	0.000	0.071	0.071
χ^2 =	11.7***	8.3***	8.3***	6.8**	9.1***	17.1 ***	9.9***	0
G =	4.8*	6.7**	6.7**	5.2*	7.1**	7.9**	7.4**	0
N_e	1.14	1.12	1.11	1.09	1.13	1.13	1.14	1.13
H_O	0.063	0.054	0.058	0.031	0.052	0.032	0.045	0.047
SD	0.156	0.112	0.133	0.065	0.116	0.078	0.112	0.128
H_E	0.08	0.08	0.07	0.06	0.08	0.09	0.08	0.07
F_{IS}	0.189	0.326	0.223	0.521	0.376	0.632	0.458	0.376
N individuals	20	14	12	16	13	10	14	15

*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; nonsignificant when P was absent.

d.f. = 4, $P < 0.01$). The homozygote BB was found to occur significantly more in deciduous woods than did the homozygote AA or to a lesser extent the heterozygote (Fig. 3). The homozygote BB in the Tyrosinase locus resulted in a dark colour phenotype, here associated with a characteristic habitat, i.e. deciduous woods. Further-

Table 2 Latitude, average frequency of five main habitat types and habitat diversity index in eight polecat populations.

	Anjou	Vendée	Brière	Brittany	Aquitaine	Morvan	Limousin	Sologne
Latitude	47.2	46.6	47.3	48	44.5	47.1	45.8	47.4
Willow groves & marshes	0.357	0.391	0.338	0.155	0.359	0.197	0.208	0.373
Deciduous woods	0.268	0.214	0.307	0.435	0.289	0.291	0.398	0.302
Coniferous woods	0.031	0.041	0.012	0.066	0.052	0.042	0.051	0.05
Cultures	0.128	0.142	0.141	0.115	0.086	0.082	0.112	0.118
Natural meadows	0.216	0.212	0.202	0.229	0.214	0.388	0.231	0.157
Habitat diversity	3.798	3.767	3.713	3.530	3.727	3.539	3.701	3.685

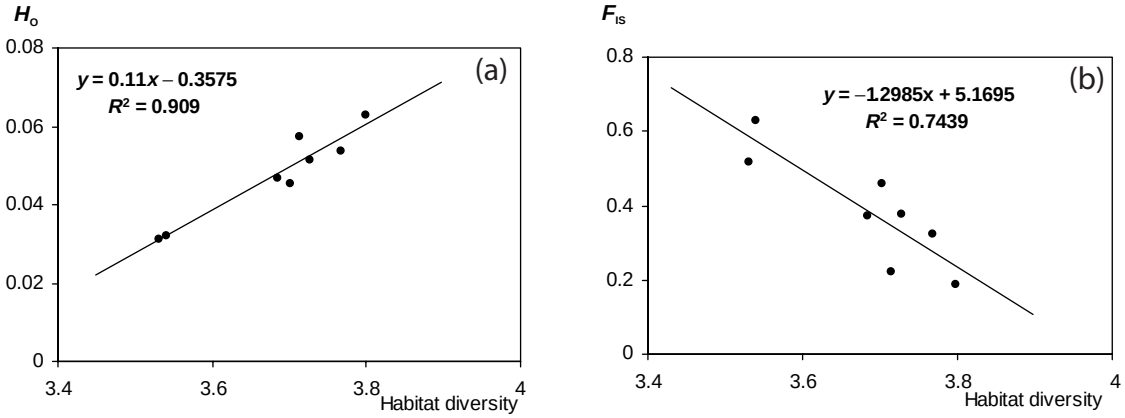

Fig. 2 Linear regression between mean observed heterozygosity (H_o) and habitat diversity index, and between F_{IS} index and habitat diversity index.

more, and although the polymorphism for the TYR-1 locus concerned seven on the eight studied populations (87.5%), the heterozygotes AB were found to be in the minority (mean frequency 7.3%) suggesting a disruptive effect.

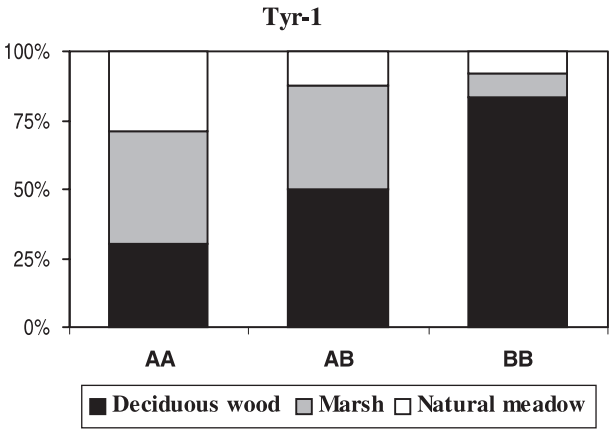

Fig. 3 Distribution of the main habitats occupied by polecats for the three genotypes of the Tyrosinase-1 locus (the homozygote BB was mainly found in deciduous woods).

Discussion

Habitat and genetic variation

Carnivorous species have been expected to have less genetic variability than other taxa (Wooten & Smith, 1985; Merola, 1994). However, polecat populations were characterized by a relatively strong allelic differentiation. In fact, several mustelid species exhibited such a genetic diversity, thus refuting this previous assumption (Hartl *et al.*, 1988; Serfass *et al.*, 1998; Lodé, 1999). In polecat, levels of heterozygosity clearly differed among populations and the deviations from Hardy–Weinberg revealed an important heterozygote deficit more pronounced in oligotrophic environments such as Morvan, Brittany and Limousin than in eutrophic environments such as Anjou or Brière. Nevertheless, the habitat composition in estimated home range showed few differences among populations and whatever the studied parameter, genetic variability was not linked to any habitat features other than diversity index.

Polecats are known to occupy various habitats alternatively or to successively exploit woods, ponds, marshes and meadows (Lodé, 1993, 1994). Their use of habitats depends upon the successive availability of distinct

trophic resources, mainly rodents and anuran (Lodé, 1997, 2000). Thus the habitat structure in their home range corresponds to habitat mosaic structure in which discrete habitat types are more or less interspersed. The habitat heterogeneity could lead to a heterosis effect. Environmental stochasticity may hamper the individual optimization of phenotypes (Moran, 1992; McNamara, 1997). The habitat mosaic structure consists of fairly stable and clear-cut environmental variation where exerted selective pressures could be contradictory. Multiple constraints could result in phenotypic plasticity corresponding to the best possible ecological compromise (Schneider *et al*., 1991; Moran, 1992; Robinson & Wilson, 1996a, b). A genetic heterozygosity correlated with habitat diversity supports the genetic prediction of a weak genetic differentiation in a stable homogeneous environment.

Disruptive selection

The large deficiency of heterozygotes may result from the social structure of the species leading to a Wahlund effect. A moderate inbreeding exists in carnivorous species living in familial packs which divide populations in small units (Hamilton & Kennedy, 1986; Evans *et al*., 1989; Kennedy *et al*., 1990). But polecats live a very solitary life based on a relatively strict territorial organization and show a clear segregation in the use of space even between males and females (Lodé, 1996). These individualistic habits resulted in a wide distribution and a family effect could not be suspected. An alternative hypothesis is that the heterozygote deficit might also be explained by the hybridization of two sibling species in this area. A more detailed study using neutral markers would be required but this explanation is doubtful because dark and typical polecats only differ in one out of 40 allozymes, show few morphological differences (excepted coat colour and size) and completely interbreed in nature (Lodé, 1995). Genetic polymorphism leading to habitat preference has been documented on some invertebrates (Jaenicke & Holt, 1991; Johannesson *et al*., 1995; Johannesson & Tatarenkov, 1997; Bush & Smith, 1998). Habitat preference often requires the effect of several loci or could be polygenic (Jaenike & Holt, 1991; Stanhope *et al*., 1993). In fact, character differentiation mainly resulted from selective association between ecological and morphological traits increasing divergence. In polecat, most of the polymorphic loci reflected allelic differentiation among populations. But, the polymorphism found in Tyrosinase was chiefly expressed as a divergence among genotypes within populations, determining the distinct phenotypes. All the phenotypes were sympatric but the rare homozygote BB governing the 'dark' phenotype was found to exploit mainly deciduous woods. The genetic basis for this character differentiation was demonstrated here in a noticeable situation without spatial isolation. Furthermore, the BB homozygote was found in seven out of eight populations although it remained uncommon. The fact that dark coat was linked to wooded habitats is still enigmatic but is also found in other carnivorous whereas mustelids inhabiting the steppe or the prairie such as the Steppe polecat *M. eversmanni* or the Black footed ferret *M. nigripes* display a light-coloured pattern (Ortolani & Caro, 1996). In any case, the question how such a minority homozygous phenotype could maintain itself should be addressed.

Sympatric differentiation is often argued for species living in a poor environment with highly contrasted resources (Abrams, 1987; Robinson *et al*., 1993; Taylor & Bentzen, 1993; Skulason & Smith, 1995; Tregenza & Butlin, 1999). For instance, polyphenotypism in Arctic charr imply both diet segregation and use of benthic, pelagic or littoral habitats (Malmquist *et al*., 1992). The polecat phenotype divergence suggested however, that more subtle differences in environment could influence genetic differentiation, a situation that is likely to be common in most natural populations.

Disruptive selection may have arisen from intraspecific competition for habitats or because of the disadvantage to intermediate genotypes. The presumption for disadvantage to intermediates was supported by the restricted number of heterozygotes for Tyrosinase found in all polecat populations. Similarly, polecats exhibit strong competitive interactions for habitat (Lodé, 1993, 1996). An alternative proposition would be that heterozygote deficit may result from an assortative mating intra phenotype, i.e. homogamy. Recurrent mutations are rarely involved in maintaining rare phenotypes (Ridley, 1996) but in sexual populations, mating with a particular phenotype may be selectively favoured, increasing the divergence between genotypes. The maintenance of the genotype BB in the Tyrosinase locus may stem from such a homogamy.

Ecological heterogeneity may have been a key-factor responsible for divergence (Schliewen *et al*., 1994). From allopatric to sympatric differentiation, adaptive variations could occur at various levels in ecological systems and induce divergence among individuals. In any case, the link between the genetic divergence within polecat populations and polecat partitioning for habitat supports the adaptive significance of habitat preference.

Acknowledgments

I thank D. Le Jacques and A. M. O'Donovan who contributed to the manuscript. Thanks are also due to K. Johannesson who provided valuable comments on the manuscript. I especially appreciated the help of C. Bourdon, M. Brégéon, I. Charissou, M. Chauvet, Association Erminea, Groupe Mammalogique du Limousin, D. Guérineau, B. Guichard, V. Hardouineau, C. Joyaux, M. Kabala, F. Ibanez G. Labidoire, M. Lambert, A. Le Beller, H. Le Corronc, S. Mazaud, D. Montfort, J. Noël,

O.N.C., P. Pailley, C. Pacteau, M. Panterne,Y. Philipeau, A. Pihuit, J. Pourreau and S. Daniel.

References

Abrams, P.A. 1987. Alternative models of character displacement (2) displacement when there is competition for a single resource. *Amer. Nat.* **130**: 271–282.

Abrams, P.A. 1990. Ecological versus evolutionary consequences of competition. *Oikos* **57**: 147–151.

Allendorf, F.A. & Leary, R.F. 1986. Heterozygosity and fitness in natural populations of animals, In: *Conservation Biology, the Science of Scarcity and Diversity* (M.E. Soulé, ed.), pp. 57–76. Sinauer, Sunderland.

Ayala, F.J. 1976. *Molecular Evolution.* Sinauer, Sunderland, MA.

Birks, J.D.S. & Kitchener, A.C. 1999. *The Distribution and Status of the Polecat Mustela Putorius in Britain in the 1990s*. The Vincent Wildlife Trust, London.

Blandford, P.R.S. 1987. Biology of the polecat *Mustela putorius*: a literature review. *Mammal Rev.* **17**: 155–198.

Blondel, J., Dias, P.C., Perret, P., Maistre, M. & Lambrechts, M.M. 1999. Selection-based biodiversity at a small spatial scale in a low dispersing insular bird. *Science* **285**: 1399–1402.

Bush, G.L. & Smith, J.J. 1998. The genetics and ecology of sympatric speciation: a case study. *Res. Popul. Ecol.* **40**: 175–187.

Butlin, R.K. & Tregenza, T. 1997. Is speciation no accident? *Nature* **387**: 551–553.

Dieckmann, U.L.F. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature* **400**: 354–357.

Endler, J.A. & Théry, M. 1996. Interacting effects of lek placement, display behavior, ambient light and color patterns in three neotropical forest-dwelling birds. *Am. Nat.* **148**: 421–452.

Evans, P.G.H., MacDonald, D.W. & Cheeseman, C.L. 1989. Social structure of the European Badgers *Meles meles*: genetic evidence. *J. Zool., London* **218**: 587–595.

Ferguson, A. & Taggart, J.B. 1991. Genetic differentiation among the sympatric bown trout (*Salmo trutta*) populations of lough Melvin, Ireland. *Biol. J. Linn. Soc.* **43**: 221–237.

Garland, T. & Adolph, S.C. 1991. Physiological differentiation of vertebrate populations. *Annu. Rev. Ecol. Syst.* **22**: 193–228.

Grant, P.R. 1975. The classical case of character displacement. *Evol. Ecol.* **8**: 237–337.

Hamilton, M.J. & Kennedy, M.N. 1986. Genic variation in the coyote, *Canis latrans* in Tennessee, USA. *Genetica* **71**: 167–173.

Hartl, G.B., Willing, R., Grillitsch, M. & Klansek, E. 1988. Biochemical variation in *Mustelidae*: are carnivores genetically less variable than other mammals. *Zool. Anz.* **221**: 81–90.

Jaenicke, J.J. & Holt, R.D. 1991. Genetic variation for habitat preference: evidence and explanations. *Amer. Nat.* **137**: 537–590.

Johannesson, K. & Tatarenkov, A. 1997. Allozyme variation in a snail (*Littorina saxatilis*) – deconfounding the effects of microhabitat and gene flow. *Evolution* **51**: 402–409.

Johannesson, K., Johannesson, B. & Lundgren, U. 1995. Strong natural causes microscale allozyme variation in a marine snail. *Proc. Natl. Acad. Sci. USA* **92**: 2602–2606.

Kennedy, P.K., Kennedy, M.L., Clarkson, P.L. & Liepins, I.S. 1990. Genetic variability in natural populations of the grey wolf, *Canis lupus*. *Can. J. Zool.* **69**: 1183–1188.

Kimura, M. & Crow, J.F. 1964. The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725–738.

Kondrashov, A.S. & Kondrashov, F.A. 1999. Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400**: 351–354.

Levins, R. 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton, NJ.

Lodé, T. 1993. Stratégies d'utilisation de l'espace chez le Putois européen *Mustela putorius* L. dans l'ouest de la France. *Rev. Ecol.* (Terre Vie) **48**: 305–322.

Lodé, T. 1994. Environmental factors influencing habitat exploitation by the polecat *Mustela putorius* in western France. *J. Zool. London* **234**: 75–88.

Lodé, T. 1995. Convergence morphologique du putois (*Mustela putorius*) et du vison américain (*M. vison*) avec le vison d'Europe (*M. lutreola*). *Gibier Faune Sauvage* **12**: 147–158.

Lodé, T. 1996. Conspecific tolerance and sexual segregation in European polecat. *Acta Theriol.* **41**: 171–176.

Lodé, T. 1997. Trophic status and feeding habits of the European Polecat *Mustela putorius* L., 1758. *Mammal Rev.* **27**: 177–184.

Lodé, T. 1998. Genetic heterozygosity in polecat *Mustela putorius* populations from western France. *Hereditas* **129**: 259–261.

Lodé, T. 1999. Genetic bottleneck in the threatened western population of European mink *Mustela lutreola. Ital. J. Zool.* **66**: 351–353.

Lodé, T. 2000. Functional response and area-restricted search of a predator: seasonal exploitation of anurans by European polecat. *Mustela Putorius. Autral. Ecol.* **25**: 223–231.

Lynch, J.M. & Hayden, T.J. 1995. Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). *Biol. J. Linn. Soc.* **55**: 293–307.

McNamara, J.M. 1997. Phenotypic plasticity in fluctuating environments: consequences of the lack of individual optimization. *Behav. Ecol.* **9**: 642–648.

Malmquist, H.J., Snorrason, S.S., Skulason, S., Jonsson, B., Sandlund, O.T. & Jonasson, P.M. 1992. Diet differentiation in polymorphic artic charr, *Salvelinus alpinus*. *J. Anim. Ecol.* **61**: 21–35.

Martin, T.E. 1998. Are microhabitat preferences of coexisting species under selection and adaptive? *Ecology* **79**: 656–670.

Mayr, E. 1963. *Animal Species and Evolution*. Harward Univ Press, Cambridge, Mass.

Merola, M. 1994. A reassessment of homozygosity and the case for inbreeding depression in the Cheetah, *Acinonyx jubatus:* implications for conservation. *Conserv. Biol.* **8**: 961–971.

Moran, N.A. 1992. The evolutionary maintenance of alternative phenotypes. *Am. Nat.* **139**: 971–989.

Murphy, R.W., Sites, J.W., Buth, D.G. & Haufler, C.H. 1990. Proteins I: isozyme electrophoresis In: *Molecular Systematics* (D.M. Hillis & C. Moritz, eds), pp. 45–126. Sinauer Press, Sunderland, MA.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Ortolani, A. & Caro, T.M. 1996. The adaptive significance of color. Patterns in carnivores: phylogenetic tests of classic hypotheses In: *Carnivore, Behavior, Ecology and Evolution* (J.L. Gittleman, ed.), pp. 132–188. Cornell University Press, New York.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. 1987. *Manuel Technique de Génétique Par Électrophorèse Des Protéines*. Lavoisier, Paris.

Pfenning, D.W. 1990. 'Kin recognition' among spadefoot toad tadpoles. A side effect of habitat selection. *Evolution* **44**: 785–798.

Ridley, M. 1996. *Evolution*. Blackwell Science Inc., Oxford.

Rice, W.R. & Hostert, E.E. 1993. Laboratory experiments on speciation – what have we learned in 40 years. *Evolution* **47**: 1637–1653.

Robinson, B.W. & Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. *Amer. Nat.* **144**: 596–627.

Robinson, B.W., Wilson, D.S., Margosian, A.S. & Lotito, P.T. 1993. Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. *Evol. Ecol.* **7**: 451–464.

Robinson, B.W. & Wilson, D.S. 1996a. Genetic differentiation and phenotypic plasticity in sympatric morphs of pumpkinseed sunfish (*Lepomis gibbosus*). *Evol. Ecol.* **10**: 1–22.

Robinson, B.W. & Wilson, D.S. 1996b. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evol. Ecol.* **10**: 631–652.

Rothe, G.M. 1994. *Electrophoresis of Enzymes, Laboratory Methods*. Springer-Verlag, London.

Schliewen, U.K., Tautz, D. & Pääbo, S. 1994. Sympatric speciation suggested by monophily of crater lake cichlids. *Nature* **368**: 629–632.

Schneider, S.M., Caplan, R.L. & Lyman, R.F. 1991. The genetics of phenotypic plasticity. III genetic correlations and fluctuating asymmetries. *J. Evol Biol.* **4**: 51–68.

Searle, A.G. 1968. *Comparative Genetics of Coat Colour in Mammals.* Logos, London.

Serfass, T.L., Brooks, R.P., Novak, J.M., Johns, P.E. & Rhodes, O.E. 1998. Genetic variation among populations of river otters in North America: considerations for reintroduction projects. *J. Mammal.* **79**: 736–746.

Skulason, S. & Smith, T.B. 1995. Resource polymorphisms in vertebrates. *TREE* **10**: 366–370.

Skulason, S., Snorrason, S.S., Ota, D. & Noakes, D.L.G. 1993. Genetically based differences in foraging behaviour among sympatric morphs of artic charr (Pisces: Salmonidae). *Anim. Behav.* **45**: 1179–1192.

Smith, T.B. 1993. Disruptive selection and the genetic basis of bill size polymorphism in the African finch *pyrenestes. Nature* **363**: 618–620.

Stanhope, M.J., Hartwick, B. & Baillie, D. 1993. Molecular phylogeographic evidence for multiple shifts in habitat preference in the diversification of an amphipod species. *Mol. Ecol.* **2**: 99–112.

Taylor, E.B. & Bentzen, P. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Smerus*) in northeastern north america. *Evolution* **47**: 813–832.

Tregenza, T. & Butlin, R.K. 1999. Speciation without isolation. *Nature* **400**: 311–312.

Willis, M.B. 1989. *Genetics of the Dog*. Whitherby, London.

Wooten, M.C. & Smith, M.H. 1985. Large mammals are genetically less variable? *Evolution* **39**: 212–215.

Wright, S. 1978. *Evolution and the Genetics of Populations IV Variability Within and Among Natural Populations*. University Press, Chicago.

Received 18 October 2000; revised 21 December 2000; accepted 10 January 2001

Supplementary material

The following material is available from http://www.blackwell-science.com/products/journals/suppmat/JEB/JEB275/JEB275sm.htm

Multiple regression analysis of three genetic parameters (mean observed heterozygosity H_o, F_{IS} index and effective number of alleles N_e) and the main habitat types and latitude of eight polecat populations.

Genotypic frequencies for 10 polymorphic locus according to different dominant habitats in polecat home ranges.