

European Mink–Polecat Hybridization Events: Hazards From Natural Process?

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Abstract

Determining the significance of hybridization events raises essential issues both in conservation and in evolutionary biology. Here, we report a genetic investigation of sympatric polecat and endangered European mink populations. Although the two species were morphologically very similar, the European mink and the polecat were easily discriminated from allozymes and microsatellites and showed a high level of private alleles (effective number of alleles: mink=1.45 and polecat=3.09). Nevertheless, the allozymic polymorphism remained lower in the European mink (4 loci, 10.5%) than in polecat (9 loci, 23.7%). Similarly, from microsatellite data, the polymorphism only reached 36% at 0.99 in the European mink; whereas in the polecat, the polymorphism reached 82% at 0.99. Natural hybridization events between two native species were detected. Because of the low fertility of hybrids, interbreeding could be regarded as producing “hybrid sink” that leads to a progressive assimilation of mink by polecat. Nonetheless, pure mink populations inhabited streams in western France, and hybridization events were only detected in areas where mink were rare and now presumed disappeared. Rather than revealing the poor efficiency of the specific recognition system, our results suggest that hybridization is associated with the scarcity of mating partners.

Documenting patterns of gene exchange between species has received growing interest as evolutionary biologists recognized that hybridization provides an exceptional set of problems to investigate reproductive isolation (Arnold et al. 1999). Hybridization may demonstrate the low efficiency of the specific mating recognition system (SMRS) (Paterson 1993; Randler 2002), illustrating incomplete sexual isolation, and may lead to considerable evolutionary changes (Grant and Grant 1994; Arnold et al. 1999). Mixing genes from different species may alter evolutionary processes and change the speed of speciation between two taxa (Arnold 1997; Grant and Grant 1994; Dowling and Secor 1997; Stone 2000). Moreover, hybridization may have dramatic effects for rare species living in sympatry with common species, especially after human-induced introductions (O'Brien and Mayr 1991; Rhymer and Simberloff 1996; Allendorf et al. 2001). When native and introduced species hybridize, the conservation of native species implies developing a plan for preventing such hybridization (Crozier 1997). Hybridization events may result in sterile offspring because of the incompatibility of genes or enzyme systems inherited from two dissimilar species. With or without introgression, such hybridization, especially after human-induced introductions, has produced the decline or the extinction of numerous

native species (Rhymer and Simberloff 1996; Allendorf et al. 2001). When mating occurs with individuals from closely related species, hybridization may not result in the entire sterility of hybrids. Usually, the mechanism leads to low hybrid fitness, but such fertile crosses between genetically distinct animals present two main concerns. First, when native and introduced species hybridize, the rare species may be particularly affected by alien genes jeopardizing the genetic pool (Sillero-Zubiri et al. 1996; Vila and Wayne 1999); and, secondly, hybridization may entail outbreeding depression (Frankham et al. 2002). Hybridization may not allocate the particular set of genes allowing the survival in a specific environment, a process known as outbreeding depression (Templeton 1989; Thornhill 1993; Storfer 1999; Frankham et al. 2002). Furthermore, populations with a small effective size are highly vulnerable to genetic depletion (Frankham et al. 2002).

While a large consideration for biological conservation has been devoted to anthropogenic incidental hybridization (Allendorf et al. 2001; Frankham et al. 2002), natural hybridization raises controversial concerns. Natural hybridization may be regarded as a natural component of the evolutionary process of related species (Arnold 1992; DeMarais et al. 1992; Arnold et al. 1999). Nevertheless, the

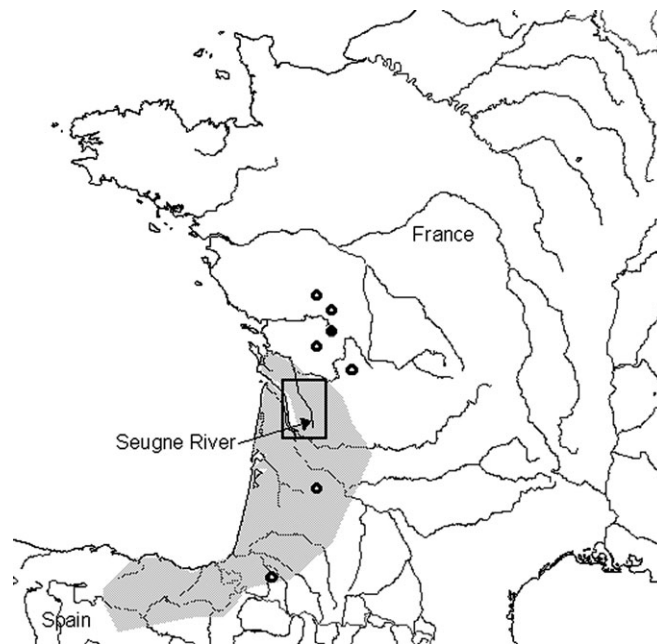


Figure 1. Sample localities of the European mink-polecat hybrid (black circle, 46°16'N, 0.03°W) and of six polecats evidencing hybridization events (white circle). Presumed current range of European mink *M. lutreola* western population (gray area: polecats live in sympatry in this area) and study area on the Seugne River are indicated.

conservation of native pure populations should be addressed when a relatively widespread species can hybridize with a rare threatened species such as the endemic European mink *Mustela lutreola*, which had a recent history of population bottleneck (Maran and Henttonen 1995; Maran et al. 1998; Lodé 1999). The European polecat *M. putorius* remained a widespread species in western Europe, although showing declining populations this species is now regarded as vulnerable. The European mink is considered as one of the most endangered species, and the western population of European mink showed a severe demographic decline (Lodé et al. 2001), with the current distribution being affected by watercourse quality and riparian habitat (Lodé 2002, Zabala et al. 2003). Allendorf et al. (2001) mentioned that potential hybridization with the introduced American mink *M. vison* has accelerated the decline of the European mink. But in western France, European mink had been declining several years before the feral American mink colonized watercourses (Lodé et al. 2001). European mink and polecats are closely related mustelids, which may be regarded as sympatric sister species (Sato et al. 2003). The dark phenotype of polecat shows a morphological convergence with the European mink (Lodé 2001a). Both species, called “water-polecats” by hunters, are often misidentified in the wild. European mink-polecat hybridization events could be regarded as a natural process concerning two sympatric native uncommon species questioning the significance of hybridization in the local extinction process. The evolutionary consequences of such natural events raise considerable issues regarding both outbreeding, the adaptive value of hybridization, and specific reproductive isolation.

We examined genetic variations between individuals morphologically identified as polecat or mink, both within a sympatric area and outside this area. Investigating natural hybridization events in two native species, our goal is: (1) to identify which alleles could be diagnostic between the two species and (2) to estimate the level of effective hybridization in sympatric populations.

Materials and Methods

Frequency of Hybridization Events

We performed a capture design, carried out on the Seugne River and adjacent watercourses (Haute-Saintonge), between 1999 and 2002 to assess the hybridization rate between the European mink and the polecat, which breed sympatrically in this area (Figure 1). This area may be regarded as the current north range of the European mink's western population after the mink decline from northwestern France (Lodé et al. 2001); only polecats are widespread outside this area. Although, as top predators, carnivores are generally found in low densities even in optimal conditions, both polecats and European mink were commonly found on the Seugne River, and the Haute-Saintonge may be one of the main mink subpopulations in the world (Lodé and Peltier 2004). Furthermore, this area should be considered as a contact zone. A total of 70 wire-mesh traps were placed every 75–100 m in two lines along a 5–6 km stretch of river for 20 consecutive nights. Live-trapping sessions were carried out 17 times (every three months) in the same study zones,

totaling 75 km of riverbank-side. Field data included sex, age, weight, date, and location of capture. A total of 35 European mink were live-trapped, and the animals were released. For genetic studies, a sample of hair was removed from 19 mink. Although dark polecats and mink can be discriminated by the length of the guard hair (<23 mm in European mink, >35mm in polecat), genetic analysis is often required in numerous cases. Both European mink and polecat start breeding at one year old, in the spring following their birth.

Genetic Determination

We investigated genetic variations in 51 European mink and 126 polecats. Crude proteins and DNA extracts were obtained from tissue samples of 32 road-killed mink and 114 polecats opportunistically sampled from the Vendée to the Pyrenees, France. In addition, we analyzed DNA from hair of 19 live-trapped individuals regarded as mink and 12 polecats in the study area. Allozymic variation was measured from 38 presumptive structural gene loci by starch gel electrophoresis, using three continuous buffer systems (TC6, TC8, TEB8), (Lodé 2001a). Slices were stained following Pasteur et al. (1987), Murphy et al. (1990), and Rothe (1994). Loci successfully resolved were: 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 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); SDH (1.1.1.14); SOD (1.15.1.1); TPI (5.3.1.1); and two nonspecific proteins. Electromorphs were presumed to have a simple genetic basis. The number 100 was assigned to the most common alleles, and other alleles were designated in increasing order of mobility.

Individuals were genotyped with 11 nucleotide microsatellites (Mvis002, Mvis020, Mvis027, Mvis054, Mvis072, Mvis075, Mvis099, Mvis111, Mvis389, Mvis1843, PutFK1) using primer sequences that allowed amplification of simple sequence repeats published for related species (O'Connell et al. 1996; Brusgaard 1997; Fleming et al. 1999) and of simple sequence repeat evidenced in *M. putorius furo* (FK1) (GB : U06156.1). DNA was extracted from muscle tissue using standard chloroform-phenol protocol (Biase et al. 2002) and from hair follicles based in the method published by Wang et al. (2002). PCR amplifications were performed in 25 µl, and amplifications were run using a thermocycler biorad with 30 s at 94°C, 30 s at T_m and 30 s at 72°C. Products were denatured, resolved on polyacrylamide gels for detection by silver staining, and sized (Peltier and Lodé 2003). Sequencing reaction ladders were used. The effective number of alleles and assignment tests (maximum likelihood method) were performed using the Genetix (Belkhir, Genetix@crit.univ-montp2.fr) and WhichRun programs (Banks M and Eichert W, <http://www.bml.ucdavis.edu/>

whichrun.htm). For each species, a value represents the likelihood of the individual's membership in that population. Each individual genotype is represented by its score for each allele at each locus.

Results

Hybridization and Discrimination between European Mink and Polecat

Only four allozyme loci (10.5%) were found polymorphic in European mink (*Est-2*, *Me-1*, *Mdb-1*, *Np*), while allozyme polymorphism was found in nine loci (23.7%) for polecat (*Ada*, *Est-2*, *G6pgdb*, *Me-1*, *Mdb-1*, *Np*, *Pep-2*, *Pgm-2*, *Sdb*) (Table 1). From allozymic variations, European mink and polecats were easily discriminated from five loci (Table 2).

Three animals had been erroneously determined. Two putative European mink were genetically identified as polecats, exhibiting the coat pattern of the “dark” phenotype. Both the length of guard hair and the genetic pattern made the determination certain. However, one heterozygous individual displayed both diagnostic alleles of European mink and polecat at five loci (Table 2). Because this animal displayed both allozymic characteristics of the two species, it should be regarded as an F1 hybrid between European mink and polecat.

From microsatellite data, polecats and European mink could be differentiated by five polymorphic and two monomorphic loci (Table 2). The loci Mvis002, Mvis020, Mvis075, and Mvis1843 clearly discriminated between European mink and polecat. The assignment test revealed these alleles to be diagnostic and confirmed one individual as a hybrid (Figure 2; probability 0.0001). The hybrid showed an intermediate pattern (Table 2). At locus Mvis054, a 118 pb allele was present in European mink, whereas a 114 pb allele was fixed in polecats. Interestingly, we also found the same 118 pb allele in three heterozygous polecats. At locus Mvis111, a small allele (89 pb) was fixed in European mink, but only two heterozygous polecats (one of which was among the previous individuals) showed the same allele. The clear-cut variation in allele size between the two species (five repeats; 10 base pairs) allowed us to exclude the possibility that the European mink allele could originate from a polecat allele by replication slippage. Similarly, the locus Mvis389 was monomorphic (103 pb) in the mink population; but two polecats (3%) fixed the same allele (103 pb), whereas every other polecat showed another allele (107 pb). Thus, for these three loci, the shared alleles were common in mink but rare and only found in heterozygous polecats. These indications were suggestive of six previous hybridization events between the two native carnivores. Most of these six heterozygous individuals—and the hybrid—were found in the north of the current range of European mink (Figure 1).

Only four loci (36%) were found polymorphic at 0.99 in European mink (Table 3); whereas in the European polecat, the polymorphism reached 82% at 0.99 with nine loci over 11 scored (Table 4). Similarly, the mean number of alleles per locus was lower in the European mink than in the polecat.

Table 1. Allele frequencies at nine polymorphic allozyme loci for European mink *M. lutreola* and polecat *M. putorius*

Polymorphic Allozyme locus		<i>M. putorius</i> N=114		<i>M. lutreola</i> N=32	
Ada	<i>Allele designation</i>	100	104	111	
	<i>Allele frequency</i>	0.535	0.465	1.00	
Est-2	<i>Allele designation</i>	100	108	96	98
	<i>Allele frequency</i>	0.141	0.859	0.133	0.866
G6pgd	<i>Allele designation</i>	100	110	100	
	<i>Allele frequency</i>	0.815	0.185	1.00	
Mdh-1	<i>Allele designation</i>	92	100	106	114
	<i>Allele frequency</i>	0.732	0.032	0.236	0.946
Me-1	<i>Allele designation</i>	100	106	112	
	<i>Allele frequency</i>	0.687	0.312	0.283	
Np	<i>Allele designation</i>	100	104	106	104
	<i>Allele frequency</i>	0.544	0.434	0.022	0.386
Pep-2	<i>Allele designation</i>	100	105	112	
	<i>Allele frequency</i>	0.926	0.074	1.00	
Pgm-2	<i>Allele designation</i>	100	102	100	
	<i>Allele frequency</i>	0.862	0.138	1.00	
Sdh	<i>Allele designation</i>	100	110	100	
	<i>Allele frequency</i>	0.532	0.468	1.00	
Mean Observed heterozygosity H_O =		0.050		0.019	

Frequency of Hybridization Events

Although the European mink was a rare and critically endangered species, a total of 35 European mink were live-trapped on 58 occasions, representing the highest number of European mink ever captured in the same area. We found no hybrid among these individuals, showing that pure European mink population currently inhabited the Seugne River. The sex-ratio of adults (number of males to number of females) only reached 0.69, emphasizing the predominance of mature females.

All live-trapped polecats (n=12) were pure polecats, thus revealing that the two native species can live sympatrically with no hybridization events being detected. Consequently, hybridization events should be considered as occurring very rarely (less than 3%, 1 vs 35 individuals), being beyond the level of detection by our design, or as happening elsewhere.

Discussion

Hybridization events between the European mink and the polecat address an original evolutionary issue about the coexistence of sympatric native species and mechanisms promoting reproductive isolation. The main results are that: (1) hybridization events between European mink and polecats were rare and only detected in areas where European mink is rare and currently presumed extinct; and (2) no hybrids were found in the Seugne River despite the high number of European mink and polecats captured.

As was expected in a bottlenecked species (Nei et al. 1975), European mink exhibited a lower allelic differentiation than polecats, both in allozymes and in microsatellites.

The effective number of microsatellite alleles per locus in polecats was twice higher than in mink (mink=1.45 and polecat=3.09). Nevertheless, the two species were easily discriminated from allozymes and microsatellites and showed many private alleles. Reviewing the power of resolution of genetic markers discriminating between species and hybrids, Boecklen and Howard (1997) noticed that using four or five markers should provide sufficient power. Here, two lines of evidences (allozymes and microsatellites) suggest that hybridization events occurred between polecats and European mink. The high level of private alleles most likely resulted from a basic divergence. By contrast, mitochondrial DNA analysis failed to resolve the relationship between polecats and European mink, revealing only one haplotype. Furthermore, that no distinct lineages were found in both species is suggestive of either a recent speciation (Davison et al. 2000) or a result of interspecific mitochondrial recent introgression (Sato et al. 2003). Little difference within species has also been demonstrated in gray wolves using mtDNA (Vila et al. 1999). Actually, the use of highly variable markers such as microsatellites should be more effective than mtDNA to allow the recognition of evolutionary significant units for conservation (Moritz 1994; Crandall et al. 2000). Because of the differential survival of the heterogametic sex or nucleocytoplasmic incompatibility, mtDNA markers should not be regarded as neutral and can be misleading when it comes to quantifying introgression.

The European mink range is actually fragmented into two population units: an eastern population unit from Ural and Estonia to the Black Sea, already subdivided into small units; and a western population (Youngman 1982; Saint-Girons 1991). The pure European mink population, which is still

Table 2. Differences in alleles discriminating European mink *M. lutreola* and polecat *M. putorius* from allozymic variations and microsatellites and alleles found in one hybrid

Allozyme locus	<i>M. lutreola</i>		<i>M. putorius</i>		Hybrid
	<i>N</i> alleles	Allele designation	<i>N</i> alleles	Allele designation	Allele designation
Ada	1	111	2	100–104	104–111
Est-2	2	96–98	2	100–108	96–100
Mdh-1	2	114–120	3	92–100–106	100–120
Me-1	2	110–116	2	100–106	100–110
Pep-2	1	112	2	100–105	105–112
Effective number of alleles per locus	1.105		1.289		
Microsatellite locus	<i>M. lutreola</i> alleles		<i>M. putorius</i> alleles		Hybrid
Mvis002	192		188–190–196–198–200–202–204		190/192
Mvis020	137		139		137/139
Mvis054	118		106–114 rare 118 in 3 polecats		114/118
Mvis075	103		105		103/105
Mvis111	089		101–103–105–107–109 rare 089 in 2 polecats		089/109
Mvis389	103		107 rare 103 in 2 polecats		103/107
Mvis1843	130–132		118–120		118/132
Effective number of alleles per locus	1.45		3.09		

relatively abundant on the Seugne River, seems to constitute a key element in defining an evolutionary significant unit for the western European mink population. Although microsatellite data suggested European mink-polecat hybridization events, only a single case evidenced such a natural hybridization in the northern range. Moreover, for three microsatellite loci, the shared alleles are common in mink but rare and only found in heterozygous polecats; thus, we may hypothesize that several hybridization events occurred, especially in the north of the current range. Actually, the occurrence of hybrids means that at least some rare mink survived until now into small, restricted populations. Although only suspected through morphological identification, the existence of wild hybrids (up to 3%) between these two species has been reported in Russia (Ternovsky 1977; Tumanov and Zverjev

1986; Maran and Henttonen 1995). Anyway, a method to identify conclusively the level of natural hybridization between polecats and European mink is required.

The low divergence between the two taxa suggested that *M. lutreola* and *M. putorius* are sister species (Sato et al. 2003). The close relationships within the polecat group (*M. putorius*, *M. furo*, *M. eversmannii*, *M. nigripes*, and *M. lutreola*) support that these species constituted a holarctic species complex, forming a *syngameon* according to Templeton's definition (1989). Experimental hybridization has established that polecats, ferrets, Steppe polecats, and European mink are able to produce fertile hybrids (Ternovsky 1977). However, natural hybridization between native species is hardly ever reported from the wild. Kit (*Vulpes macrotis*) and swift fox (*V. velox*) populations were thought to hybridize (Mercure et al.

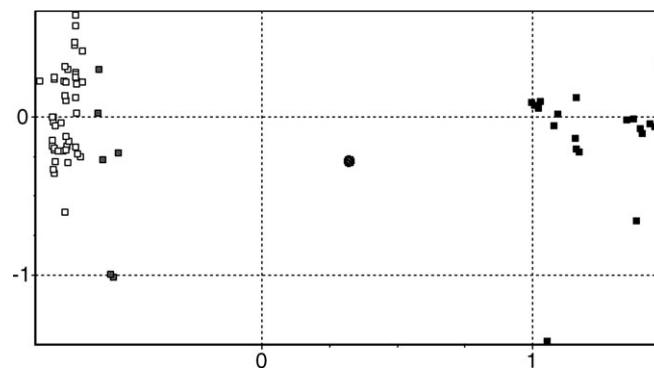
**Figure 2.** Result of assignment test based on nine microsatellite loci for European mink *M. lutreola* (black squares), polecats *M. putorius* (white and gray squares), and one hybrid (gray circle), sampled in western France. (Gray squares indicate heterozygote polecats possessing at least one mink allele).

Table 3. Allele frequencies at 11 European mink *M. lutreola* microsatellite loci

Microsatellite locus	<i>M. lutreola</i> N=51		
Mvis002	Allele designation	192	
	Allele frequency	1.00	
Mvis020	Allele designation	137	
	Allele frequency	1.00	
Mvis027	Allele designation	107	109
	Allele frequency	0.15	0.85
Mvis054	Allele designation	118	
	Allele frequency	1.00	
Mvis072	Allele designation	252	258
	Allele frequency	0.708	0.042
Mvis075	Allele designation	103	
	Allele frequency	1.00	
Mvis099	Allele designation	169	
	Allele frequency	1.00	
Mvis111	Allele designation	89	
	Allele frequency	1.00	
Mvis389	Allele designation	103	
	Allele frequency	1.00	
Mvis1843	Allele designation	130	132
	Allele frequency	0.937	0.063
PutFK1	Allele designation	149	151
	Allele frequency	0.437	0.562
Mean Observed heterozygosity $H_O =$		0.095	

1993), and genetic introgression was described between *Martes martes* and *M. zibellina* (Davison et al. 2001). The debated case of the red wolf *Canis rufus* seems also to originate from natural hybridization between coyote and gray wolf (Wayne and Jenks 1991; Nowak 1992; Reich et al. 1999). Mate recognition mechanisms could significantly contribute to prezygotic isolation and, questioning the significance of Mayr's biological concept of species, Paterson (1993) proposed that the evolutionary event for speciation first originated from a new mate recognition system while other mechanisms reducing interbreeding were incidental consequences. The hybridization may be favored by convergence in mating recognition system. The reason why European mink could hybridize with polecats may be found in phenotypic convergence. Polecats display a sympatric divergence, differentiating a "typical" morphotype from a "dark" phenotype; and the dark animals were both smaller and associated with forest brooks (Lodé 2001a), a habitat also selectively used by the European mink (Zabala et al. 2003). Nonetheless, here the rarity of hybridization events in European mink suggests that change or convergence in mating behaviors was poorly efficient to facilitate interbreeding.

When they hybridize with domestic species, endangered wild species may suffer from outbreeding depression, losing their specific adaptation to peculiar environmental constraints (Frankham et al. 2002). Hybridization with feral dogs exposed wolves (Vila and Wayne 1999) and threatened populations of Ethiopian wolves (*Canis simensis*) to a loss of their original genetic adaptations (Gottelli et al. 1994; Sillero-

Zubiri et al. 1996). Furthermore, in carnivores, females are highly philopatric, but males are often closely related (Gompper et al. 1998; Lodé 2001b). Thus, the mating system of mustelids, in which mating in adjacent home-range is favored, is found poorly efficient to retain genetic diversity (Lodé 2001b). The natural interspecific hybridization between the native polecat and the endangered mink may entail outbreeding depression because hybrids were less fertile (Tumanov and Zverjev 1986). Thus, as suggested by the fact that no mink with polecat alleles were found, hybridization may result in a progressive assimilation of European mink by polecats, thus producing a "hybrid sink," especially when hybrids or backcrosses can not transmit mink alleles. The fact that hybrids showed many microsatellite "polecat" alleles supports this hypothesis. But occurring through an apparent natural process, it may be argued that hybridization may not be a problem (Allendorf et al. 2001), especially if hybrids are fertile. Arnold et al. (1999) demonstrated that natural hybridization, by introducing new genetic diversity into an endangered population, increased the average fitness of the individuals. Alternatively, hybridization may result in extinction when there is no habitat differentiation between taxa (Wolf et al. 2001).

Actually, our results suggest that hybridization may be linked to the mink population bottleneck. If hybridization results from a low efficient SMRS, many hybrids should be found when the species breed in the same habitat. On the Seugne River, the two native species bred sympatrically, with no hybridization events. Alternatively, if hybridization results from a lack of mating partners, hybrids should be more rare

Table 4. Allele frequencies at 11 European polecat *M. putorius* microsatellite loci

locus	<i>M. putorius</i> N = 126							
Mvis002	<i>Allele designation</i>	188	190	196	198	200	202	204
	<i>Allele frequency</i>	0.587	0.036	0.073	0.022	0.116	0.152	0.015
Mvis020	<i>Allele designation</i>	139						
	<i>Allele frequency</i>	1.00						
Mvis027	<i>Allele designation</i>	107	109					
	<i>Allele frequency</i>	0.101	0.899					
Mvis054	<i>Allele designation</i>	106	114	118				
	<i>Allele frequency</i>	0.037	0.941	0.021				
Mvis072	<i>Allele designation</i>	248	250	252	254	256		
	<i>Allele frequency</i>	0.123	0.022	0.536	0.196	0.123		
Mvis075	<i>Allele designation</i>	105						
	<i>Allele frequency</i>	1.00						
Mvis099	<i>Allele designation</i>	167	169					
	<i>Allele frequency</i>	0.406	0.593					
Mvis111	<i>Allele designation</i>	89	101	103	105	107	109	
	<i>Allele frequency</i>	0.014	0.021	0.229	0.357	0.221	0.157	
Mvis389	<i>Allele designation</i>	103	107					
	<i>Allele frequency</i>	0.014	0.986					
Mvis1843	<i>Allele designation</i>	118	120					
	<i>Allele frequency</i>	0.986	0.014					
PutFK1	<i>Allele designation</i>	145	147	151				
	<i>Allele frequency</i>	0.329	0.023	0.648				
Mean Observed heterozygosity $H_O =$		0.246						

and rather present in areas where at least one of the two species is scarce. In western France, mink and polecats hybridized in areas where mink were scarce, thus supporting this second hypothesis. When a species is rare, individuals may be unable to mate within their own species, thus enlarging the opportunity for mating with individuals from closely related species. It may be suspected, then, that hybridization between polecats and European mink may proceed more and more frequently as European mink decline.

Conclusion

Natural hybridizations occurred very rarely, beyond the level of detection by our capture design. Pure European mink and polecat populations still inhabited streams in western France, and hybridization events were only detected in areas where European mink were found to be very scarce. Thus, in western France, the two species have remained genetically distinct. Our results suggest that the hybridization events may be attributable to the scarcity of mating partners resulting from the severe bottleneck in European mink populations.

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