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Genetic diversity and integrity of German wildcat (*Felis silvestris*) populations as revealed by microsatellites, allozymes, and mitochondrial DNA sequences

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Abstract

As a consequence of persecution and habitat fragmentation, wildcats (*Felis silvestris silvestris*) in Western Europe have experienced a severe reduction in population numbers and sizes. The remaining wildcat populations are considered to be endangered by losses of genetic variability and by hybridisation with free-ranging domestic cats. To investigate genetic diversity within and among wild and domestic cat populations in Germany and to estimate the extent of gene flow between both forms, we analysed a total of 266 individuals. PCR-amplification and sequencing of 322 base pairs of a highly variable part of the mitochondrial control region (HV1) of 244 specimens resulted in 41 haplotypes with 31 polymorphic sites. Additionally, eight microsatellite loci were examined for those 244 cats. Moreover, a total of 46 wildcats and 22 domestic cats could be genotyped for 13 polymorphic out of 31 enzyme loci. Genetic variability in both groups was generally high. Variability in domestic cat populations was higher than in wildcat populations. Almost no differentiation between domestic cat populations could be found (F_{ST} for microsatellites = 3%). In contrast, wildcat populations differed significantly from one another (F_{ST} for microsatellites = 9.55%) Within the smaller wildcat populations, a reduction of genetic diversity was detectable with regard to the nuclear DNA. Wildcat and domestic cat mitochondrial haplotypes were separated, suggesting a very low level of maternal gene flow between both forms. In microsatellites and to a somewhat lesser extent in allozymes, wildcats and domestic cats showed distinct differentiation, suggesting an only low extent of past hybridisation in certain populations. The microsatellite data set indicated a significantly reduced effective population size (bottleneck) in the recent past for one German wildcat population.

Keywords: *Felis silvestris*; Domestic cat; Hybridisation; Microsatellites; mtDNA

Introduction

Owing to persecution and habitat loss, the European wildcat (*Felis silvestris silvestris*) has experienced a severe reduction in population numbers and sizes. In some countries this subspecies of wildcat is already extinct, in others the remaining populations are small and fairly isolated (Piechocki 1990). The genetic integrity of wildcat populations is considered to be threatened both by the loss of allelic variation due to genetic drift and by the introgression of genes from roaming or feral domestic cats (*Felis silvestris* f. catus; Stahl and Artois 1991). While

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hybrids between wildcats and domestic cats have been observed throughout Europe, the actual extent of interbreeding is under discussion and may in fact show considerable local differences. For example, a high level of hybridisation was found in Scottish wildcats (Hubbard et al. 1992; Beaumont et al. 2001), but only two per cent of the Belgian population were considered to be hybrids (Parent 1974). The amount of gene flow between wildcats and domestic cats in Italy was estimated to be rather low and introgression proved to be geographically restricted (Ragni and Randi 1986; Randi et al. 2001). Recent studies based on microsatellites, including samples from Italy, Belgium, Portugal, Germany and Hungary, revealed extensive hybridisation only in Hungary (Pierpaoli et al. 2003; Lecis et al. 2006; Randi 2007; Oliveira et al. 2008a, b, O'Brien et al. 2009).

In Germany there are comparatively large natural populations in the Harz region, the Eifel region and in Pfälzer Wald, each of them harbouring more than 200 wildcats (Piechocki 1990; Raimer 1994). In addition there are a few small and isolated populations, some of them consisting of no more than 30 individuals (e.g. Solling, Hochtaunus, Hainich, Knüll). In these populations, genetic depletion and hybridisation might be higher than in the larger ones. Former studies on German wildcats were limited to microsatellite analysis of one or two populations and only a small number of domestic cats (Hille et al. 2000; Pierpaoli et al. 2003). The present study aims at characterising genetic diversity within and among six German wildcat populations. A genetic comparison with domestic cats from largely the same geographic areas was carried out to estimate the extent of interbreeding between both forms. To evaluate the impact of female migration relative to that of both sexes sequences of the mitochondrial DNA control region, representing a maternally inherited marker, as well as a set of microsatellites, representing a nuclear genome marker subjected to recombination, were analysed. For a fraction of those samples data on allozyme variation were included, representing a second - somewhat more conservative – nuclear gene pool compartment. Wildcats from Bulgarian populations dwelling under more natural conditions than in Germany (Zimina 1962) served as a reference group.

Material and methods

Samples

A total of 96 European wildcats and 148 domestic cats were analysed at the DNA level (see also Fig. 1). Wildcat samples came from the three large German populations Harz (n = 20), Eifel (n = 12) and Pfalz (n = 8), from the small German populations Solling (n = 12), Hainich National Park (n = 6) and Taunus (n = 8), and from three Bulgarian populations (Bulgaria 1: near Sliven, n = 8, Bulgaria 2: near Plovdiv, n = 7, and Bulgaria 3: near Radomir, n = 2). Rural domestic cats were sampled from Schleswig-Holstein (n = 28), Harz, Hainich, Solling (n = 26), Eifel (n = 24), Pfalz (n = 21), and Taunus (n = 13). An additional sample was obtained from feral cats in Berlin (n = 25). To increase sample sizes for comparison of wild and domestic cat gene pools, 13 wildcats of uncertain origin (Harz or Solling) and 11 domestic cats from either Eifel or Pfalz were also screened. Wildcat samples consisted of frozen liver tissue obtained from road kills and, in the case of Hainich National Park, of skin. Samples of domestic cats consisted mainly of shed hairs and blood. Prior to genetic analyses, all wildcats except those from Hainich had been determined as such on the basis of coat colour patterns, intestine length, and skull volume (cf. Schauenberg 1969, 1977). Moreover, a mixed sample of stray domestic cats and pet cats from Vienna (n = 22) was available for allozyme electrophoresis only.

DNA-Isolation

Total DNA was extracted from liver tissue and blood samples using the Super Quick Gene DNA isolation kit (AGTC, Analytical Genetic Testing Center, Denver, USA) according to manufacturers' instructions. The DNA was dissolved in a final volume of 100 µl Tris (pH 8,5). DNA from skin and hair samples was at first isolated by the following procedures: 5-30 hairs or a small piece of skin were incubated overnight at 55 °C in 450 µl digestion buffer (100 mM Tris pH8, 10 mM EDTA, 100 mM NaCl, 0,1% SDS) plus 40 µl dithiothreitol and 30 µl proteinase K (10 mg/ml). DNA was extracted by a phenol-chloroform-extraction (Sambrook et al. 1989) with one extraction at an equal volume of phenol-chloroform (1:1), centrifugation at 8,000 rpm for 5 min., and one extraction of the aqueous layer at an equal volume of chloroform. DNA was isolated by ethanol precipitation and dissolved in 50 µl of Tris (pH 8,5). Later on, some of the hair samples were treated with the "Dneasy Tissue Kit" from Qiagen following the manufacturers' handbook, but using the aforementioned digestion buffer.

Mitochondrial DNA analysis

1-5 µl DNA solution were used to amplify a part of the mitochondrial control region by means of the polymerase chain reaction (PCR). Double-stranded PCR amplifications were performed in a 75-µl reaction volume containing 10 mM Tris-HCl, pH 9, 50 mM KCL, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 50 µM of each dNTP and 1.5 units Taq polymerase (Appligene). The first primers we used were Lf 15926