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Original Investigation

Genetic analysis of Eurasian otters (*Lutra lutra*) reveals high admixture in Finland and pronounced differentiation in Sweden

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ABSTRACT

A number of mammal species in Europe, including the Eurasian otter (*Lutra lutra*), have experienced a decline in population size in the 20th century due to persecution, environmental pollution and ongoing habitat fragmentation. This has often led to a substantial loss of genetic diversity which may threaten population viability. While otters have been studied in some detail genetically, the northern part of the Fennoscandian range has not been covered well so far. By explicitly focussing on the genetics of otter populations from northern Sweden and Finland we aimed at closing that gap. To infer their genetic structure and diversity, we analysed sequences of the mitochondrial control region and 12 nuclear microsatellite markers in 197 Eurasian otters from Sweden and Finland. Variability of the mitochondrial control region was low overall but still revealed previously undetected haplotypes unique to the Finnish otter population. Expected heterozygosities in Fennoscandia were within the range previously reported.

Bayesian cluster analysis of our microsatellite data revealed genetic structuring of the Swedish otter populations. In contrast, we observed a high degree of admixture among the Finnish populations that we also found at the geographic border of the two countries (Lapland).

Admixed ancestry in Finnish otters suggests that gene flow from the Swedish to Central European populations is potentially facilitated via the Finnish otter populations connecting the Swedish animals with otter populations in mainland Europe.

Introduction

One key feature to ensure population viability is to maintain genetic variability within populations, this is most efficiently done by ensuring migration, which will lead to genetic exchange between populations and thus contribute to their variability (e.g. Frankham et al. 2004; Allendorf and Luikart 2007). In turn, habitat fragmentation due to spatially disconnected suitable territories or migration barriers (e.g. motorways) can have detrimental effects on genetic variability. In the worst case, the concomitant high levels of genetic drift and local inbreeding can result in genetic depletion and inbreeding depression (Zachos et al. 2007; Johnson et al. 2010). It is therefore necessary to evaluate both the genetic variability and the degree of differentiation and connectivity among populations to determine the current status and future potential viability of a species.

A number of mammalian carnivore species across Europe (e.g. *Mustela lutreola*: Maran et al. 2011; *Vormela peregusna*: Tikhonov et al. 2008; *Lynx pardinus* and *Lynx lynx*: Von Arx and Breitenmoser-Wursten 2008; Schmidt et al. 2011; *Canis lupus*: Randi 2011; *Ursus arctos*: Swenson et al. 2011) have suffered from a decline in population size. Among them is the Eurasian otter (*Lutra lutra*), a species widely distributed across the Palaearctic and reaching also the Asian Tropics. It is particularly vulnerable to anthropogenic influences such as water pollution (Olsson and Sandegren 1991) but also to other human activities, for example fishing with fyke or drift nets (Ruiz-Olmo et al. 2008). As a result, local extinctions or severe declines in population size have been documented until the 1980s (Randi et al. 2003; Elmeros et al. 2006; Stanton et al.

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2009). Fortunately, very recently numbers have increased again due to successful conservation measurements such as improved habitat quality or captive breeding programmes (Ferrando et al. 2008; Stanton et al. 2009; Koelewijn et al. 2010; Honnen et al. 2011; Roos et al. 2012). A decade ago, the species status was evaluated as "Vulnerable" in the IUCN Red List of Threatened Species, but since a re-evaluation in 2004 the otter has been classified as "Near Threatened" (Ruiz-Olmo et al., 2008). Although there is no imminent extinction risk, conservation of the species is still an issue, and regional restocking programmes have been carried out to help the species recolonise parts of their former distribution range, e.g. in the Netherlands (Koelewijn et al. 2010), Spain (Ferrando et al. 2008) and England (Stanton et al. 2009), but also in Sweden where 47 otters from Northern Norway and seven captive-bred otters were released in two regions of central Sweden (Uppsala län, seven individuals; Södermanlands län, 47 individuals) between 1987 and 1992 (Sjöåsen, 1996). Within its distribution range, the Eurasian otter is a well-studied flagship species (e.g. Cassens et al. 2000; Pertoldi et al. 2001; Mucci et al., 2004, 2010; Kalz et al. 2006; Lanszki et al. 2010). The most detailed genetic study to date covered large parts of Europe also considering the Fennoscandian populations (Mucci et al., 2010). Yet, particularly the northern parts of Finland and Sweden lacked comprehensive sampling for genetic analyses in that study.

Lapland is a cultural region that covers the Northern parts of Sweden and Finland and is the connecting area between Scandinavia and the European mainland. It is mostly located north of the Arctic Circle with short summers, while winters are long and dark (restricting the growing and breeding seasons) with continuous ice cover of water bodies. This causes seasonally scarce food supply, especially for species relying on aquatic prey (Sulkava et al. 2007). A number of generalist species are not able to expand their distribution range into this area (e.g. Wild boar and Red deer). The White-tailed Sea Eagle (*Haliaeetus albicilla*), another predator largely dependent on aquatic prey, cannot sustain large population sizes in this environment (Cederberg et al. 2003). In this bird species, a pronounced genetic differentiation among Finnish populations has been found (Ponnikas et al. 2013). The distribution of the Eurasian otter stretches into this area. Considering that Lapland connects Scandinavia with the European mainland via Russia and that the harsh environment may keep population sizes small – Central Finland: 52 individuals (study range: 1650 km^2, Sulkava et al. 2007); Finnish Lapland: 450–500 individuals (study range: 95,000 km^2, Sulkava and Sulkava, 2009) – one would expect low genetic variability and a pronounced population differentiation. Furthermore, ongoing habitat fragmentation due to road construction and forest management could strongly influence the dispersal of otters and thereby hinder gene flow resulting in decreased variability and increased differentiation, thus reducing the viability of the species in Northern Europe. Contrary to these expectations Mucci et al. (2010) only found three large genetic clusters in Fennoscandia: (i) south-west Norway; (ii) north and central Norway/central and southern Sweden; and (iii) northern Sweden and Finland. However, particularly the last grouping was not represented well in their study, with only few samples from northern Sweden and none at all from northern Finland.

In the present study, we therefore analysed 197 Eurasian otters sampled throughout Sweden and Finland with an explicit focus on the Lapland region (northern Sweden and northern Finland). Samples were analysed by means of sequencing a fragment of the mitochondrial control region and genotyping the specimens at 12 nuclear microsatellite loci. In particular, we wanted to test whether Swedish and Finnish otters exhibit as little substructuring as indicated by previous results based on limited geographic coverage (Mucci et al., 2010) or whether the genetic pattern in otters from this northern part of the distribution range is more complex than previously thought.

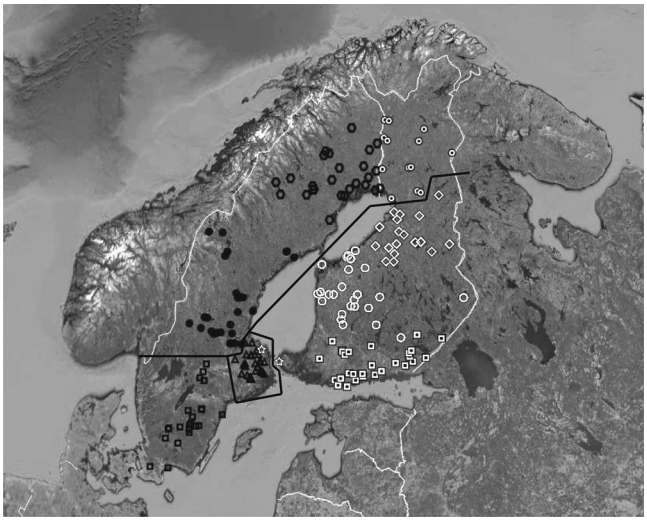

Fig. 1. Map of sampling locations. Black open squares (SSW, $N = 29$), open triangles (CESW, $N = 30$), dots (CWSW, $N = 28$) open hexagons (NSW, $N = 22$) represent individuals sampled In Sweden. White open squares (SF, $N = 25$), donuts (CF, $N = 32$), diamonds (NF, $N = 18$), dots (LF, $N = 13$) denote Finnish individuals. The two stars represent the island specimen from Gräsö and Åland. Black lines denote clusters detected by the Structure programme ($K = 4$).

Material and methods

Sampling

Otters found dead as by-catches in fishing gear or on roads were collected (2001 to 2010 in Sweden, $n = 107$; 1983 to 2009 in Finland, $n = 90$) and frozen. Two samples from islands in the Baltic Sea between Sweden and Finland (Gräsö and Åland) were also included to infer their origin. Sample locations are given in Fig. 1. An approximately 1×1 cm piece of muscle tissue was dissected and preserved in 70% ethanol for genetic analyses. DNA was extracted with the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) according to the manufacturer's protocol.

MtDNA analysis

The mitochondrial control region was amplified using the primer pair DLH (5′-CCTGAAGTAAGAACCAGATG-3′; Tiedemann et al., 1996) and ProL (5′-CACCACCAACACCCAAGCT-3′; Kocher et al., 1989) and following PCR conditions described in Cassens et al. (2000), with a reduced annealing temperature of 53 °C. The PCR products were sequenced on an automated sequencer (3730xl DNA Analyzer, Applied Biosystems, Carlsbad, CA, USA). Electropherograms were corrected visually and aligned using BioEdit version 7.8.9.0 (Hall, 1999). The data set was collapsed into haplotypes with the FaBOX package (Villesen, 2007; http://www.birc.au.dk/fabox/). Amplification and sequencing were repeated in cases of ambiguous results.

We obtained a fragment of 345 bp for 181 individuals. To integrate the detected haplotypes into the documented diversity of the control region of the Eurasian otter, 13 haplotype sequences (Lut1–Lut13, accession no.: AJ006174–78, EU294255, EU294258, FJ971618–22, HQ113947) were downloaded from GenBank. Sequences were aligned and then cut to equal length (255 bp) which allowed us to also include two additional individuals from the Fennoscandian data set (total number of individuals then 183). A median-joining network, as implemented in the Network