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Original Investigation

Admixture of two phylogeographic lineages of the Eurasian beaver in Poland

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ABSTRACT

The Eurasian beaver (*Castor fiber*) represents an uncommon example of an endangered species in which the restoration programs proved a spectacular success and led to enormous spatial and demographic expansion. Documented reintroduction of beavers in Poland has been conducted using animals of the eastern European origin, most likely derived from the eastern mtDNA lineage. However demographic and spatial expansion of beavers from Germany, which represent the western lineage, may have led to admixture of these two genetically distinct entities in Poland. We detected significant genetic differentiation between the populations from W and NE Poland both in mitochondrial DNA control region and microsatellites, but also substantial admixture including apparent first-generation migrants between regions. Our results indicate that beavers from the western mtDNA lineage have contributed considerably to the Polish population, particularly in W Poland. As there have been no adequately documented translocations of beavers from the western European populations to Poland, the observed situation appears to result from natural migration or range expansion from the west. In contrast to previous findings we detected a substantial diversity in mtDNA control region, which indicates that either the variation in relict populations has been underestimated, or that additional relict beaver populations survived at the end of the 19th century in Poland and Germany as indicated by considerable similarity of ancient and extant mtDNA haplotypes. The implications of our findings for beaver conservation and management are discussed.

Introduction

One of the consequences of range expansion may be a secondary contact between genetically differentiated populations. If these are not substantially reproductively isolated, admixed populations will form. Such zones of secondary contact have been described in many areas, and in temperate regions are often interpreted as a consequence of postglacial expansion of genetically differentiated populations from separate glacial refugia (Taberlet et al. 1998; Avise 2004; Hewitt 2004; Hofreiter and Stewart 2009; Shafer et al. 2010). Some of these contact zones may actually be hybrid zones formed between partially reproductively isolated incipient species, but many zones are relatively broad and thus unlikely to be maintained by strong selection against hybrids (Avise 2004; Abbot et al. 2013). Paleophylogeographic data suggest that such zones of secondary contact may by transient, because a thorough mixing of populations and the loss of phylogeographic structure over the expansion areas occurred in some species during the last interglacial (Hofreiter et al. 2004). Secondary contact and admixture between genetically differentiated populations may also occur during biological invasions (Kolbe et al. 2008; Keller and Taylor 2010). Genetic consequences of range expansions have recently received considerable attention (Currat et al. 2008; Excoffier et al. 2009; Petit and Excoffier 2009).

The Eurasian beaver (*Castor fiber*) represents an uncommon example of a species in which the dynamics of recolonization and its genetic consequences may be traced almost in real time. Particularly interesting in this context is the situation in the regions where genetically differentiated populations representing distinct evolutionary significant units (ESU *sensu* Moritz (1994) and Durka et al. 2005) meet during expansion. The territory of present-day Poland is an area where such a contact zone may form and admixture follow.

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The historical range of the beaver encompassed most of northern Eurasia (Djoshkin and Safonow 1972). In historical times, the species has been driven to the verge of extinction due to overhunting for fur and castoreum. At the end of the 19th century, when modern conservation measures were initiated, the species survived only in eight regions widely spaced throughout its historical distribution from France to Eastern Siberia and Mongolia (Nolet and Rosell 1998). Skull morphometric analyses provided some support for the subspecific status of these relict populations (Frahnert 2000). The total census size at that time was estimated at ca. 1200 animals, with some regional populations as small as 30 individuals (Rosell et al. 2012 and references therein). Genetic analyses of the beavers from these eight regional populations subspecies demonstrated very low genetic variation within populations and substantial differentiation among populations (Babik et al. 2005; Ducroz et al. 2005; Durka et al. 2005). Phylogeographic analysis detected two major mitochondrial (control region mtDNA) lineages (haplogroups): a western lineage composed of populations from France, Germany and Scandinavia, and an eastern lineage made up of populations from the remaining part of the beaver range. The status of separate ESU was proposed for western and eastern populations (Durka et al. 2005) as they are characterized by reciprocal monophyly of mtDNA lineages (*sensu* Moritz 1994). Based on sequence divergence, these mtDNA lineages diverged ca. 100–360 kya (Durka et al. 2005; Horn et al. 2011) and western and eastern populations are probably derived from separate glacial refugia. Analysis of ancient DNA indicates that the territory of the present-day Poland has been the area of the contact between the eastern and western clade for several thousand years, with spatial, though not temporal overlap detected at the level of single localities (Horn et al. 2014).

For several decades the beaver has been rapidly colonizing its former historical range, both naturally and with the aid of successful translocations; the current census population size exceeds one million individuals (Rosell et al. 2012 and references therein). In some areas translocations led to substantially admixed populations, such as those on the upper Danube in Bavaria and Austria which were, founded from animals originating from both eastern and western mtDNA lineages (Schwab and Lutschinger 2001). No adverse effects of such mixing, e.g. outbreeding depression, have been reported, though the possible consequences of mixing have been discussed extensively (Halley 2011; Rosell et al. 2012). The enormous spatial and demographic expansion of the species has been claimed as one of the most successful reintroductions, it is however intriguing, as the species has undergone a severe bottleneck in recent past and thus could have lost its evolutionary potential (Avise 2004).

It is currently unknown whether the beavers that inhabited Poland before extirpation represented the eastern, western or both mtDNA lineages that correspond to two different ESU *sensu* Durka et al. (2005). The species was almost extinct in Poland at the end of the World War II, and populations have been re-established by both natural immigration from Lithuania and Belorussia and by translocations of beavers imported from western Russia (Żurowski 1979, 1980, 1992; Dzięciołowski and Goździewski 1999), most probably belonging to the eastern mtDNA lineage (but see Senn et al. 2014). It was also argued that translocations from Germany occurred during the World War II, although these claims have been poorly documented (Halley and Rosell 2002). Currently Poland is inhabited by ca. 30–40 thousand beavers (Rosell et al. 2012 and references therein), occurring throughout the country, but the most numerous populations are present in NE, SE and W Poland (Czech 2010). Based on historical records and documented translocations, beaver populations inhabiting Poland are assumed to represent the eastern mtDNA lineage but are derived from multiple regions, whose relative contributions remain unknown. Ancient DNA data of Horn et al. (2014) indicate however that probably both lineages inhabited Poland in historical times. The natural expansion of beaver populations from Germany, which belong to the western mtDNA lineage, may have led to secondary admixture of these two genetically distinct groups in Poland.

In the present study we use mitochondrial DNA, autosomal microsatellites, and Y chromosome markers to: (i) assess the genetic structure of the Polish beaver populations with emphasis on the differentiation between two regions: NE and W Poland, currently sustaining the largest populations; (ii) estimate genetic variation in the Polish beaver populations; (iii) estimate the extent of admixture between eastern and western populations using multiple classes of DNA markers.

Material and Methods

The tissues for genetic analysis were collected between 2009 and 2011 in NE Poland: Podlaskie ($N = 30$) and Warmińsko–Mazurskie ($N = 8$) voivodships during standard translocation procedure of beavers within Poland constituting a part of the Programme of Active Beaver Protection. Samples from W Poland (Lubuskie voivodship) were collected from individuals legally culled to control the beaver population. Precautions were taken to not sample multiple individuals within family groups. Additionally, we obtained four samples from museum specimens: three from the Silesia region and one from Pomerania (Fig. 1). In total, we collected 91 samples that were stored in 96% alcohol until DNA extraction. We obtained multilocus microsatellite genotypes for 77 samples, while from mtDNA, sequences were aligned for 65 beavers.

Molecular analyses

DNA was extracted from dried skin fragments or ethanol-preserved tissue using the NucleoSpin Tissue Kit (Macherey and Nagel, Dueren, Germany) according to the manufacturer's protocol. Individual samples were genotyped at eight microsatellite loci (*Cca4*, *Cca5*, *Cca8*, *Cca9*, *Cca10*, *Cca13*, *Cca15*, *Cca18*) developed for *Castor canadensis* Canadian beaver (Crawford et al. 2008). All reactions were carried out with the use of Qiagen Multiplex PCR kit (Qiagen Ltd., Crawley, United Kingdom) with one primer of each pair fluorescently labeled. Reactions were carried out with 3.5 mM of $MgCl_2$ in two sets differing in the annealing temperature (Cca4-HEX, Cca 5-HEX, Cca8-FAM at 61 °C; Cca9-HEX, Cca10-FAM, Cca13-FAM, Cca15-TAMRA, Cca18-HEX at 59 °C). The reaction conditions were as follows: 15 min at 95 °C followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C/59 °C for 60 s, extension at 72 °C for 60 s and the final elongation for 10 min at 72 °C. Amplification products were resolved on an ABI PRISM 3130xl Genetic Analyser (Applied Biosystems, Foster City, USA) and sized with the internal standard LIZ 500 using the program Genemapper v.4.0 (Applied Biosystems).

A 490 bp fragment of mtDNA control region was amplified and sequenced in a subset of 65 samples representing all four sampled regions (31 from NE Poland, 30 W Poland, 3 Silesia and 1 Pomerania). We used universal primers Thr-L15926 and DL-H16340 (Cheney 1995) to amplify the hypervariable domain I (Saccone et al. 1987) of the control region (CR). This mtDNA fragment was used in previous phylogeographic analyses of the beaver and amplified according to Ducroz et al. (2005) and Durka et al. (2005).

Ten samples from the following subspecies: *C. f. albicus* (western ESU), *C. f. vistulanus*, *C. f. belorussicus*, *C. f. birulai* and *C. f. pohlei* (eastern ESU) were also analyzed for DBY7 and UTY11 markers. One to three samples were sequenced in each subspecies. The