Mammalian Biology 79 (2014) 287–296

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Original Investigation

Admixture of two phylogeographic lineages of the Eurasian beaver in Poland

Aleksandra Biedrzycka[a,*], Magdalena Konior[b], Wiesław Babik[b], Magdalena Świsłocka[c], Mirosław Ratkiewicz[c]

[a] Institute of Nature Conservation, Polish Academy of Sciences, Al. Mickiewicza 33, 31-120 Kraków, Poland
[b] Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland
[c] Institute of Biology, University of Białystok, Swierkowa 20B, 15-950 Białystok, Poland

ARTICLE INFO

Article history:
Received 19 November 2013
Accepted 25 April 2014
Handled by Paul Grobler
Available online 5 May 2014

Keywords:
Castor fiber
Expansion
Genetic admixture
Phylogeographic lineages
Reintroduction

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.

* Corresponding author. Tel.: +48 12 370 35 51; fax: +48 12 632 24 32.
E-mail address: biedrzycka@iop.krakow.pl (A. Biedrzycka).

http://dx.doi.org/10.1016/j.mambio.2014.04.005
1616-5047/

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

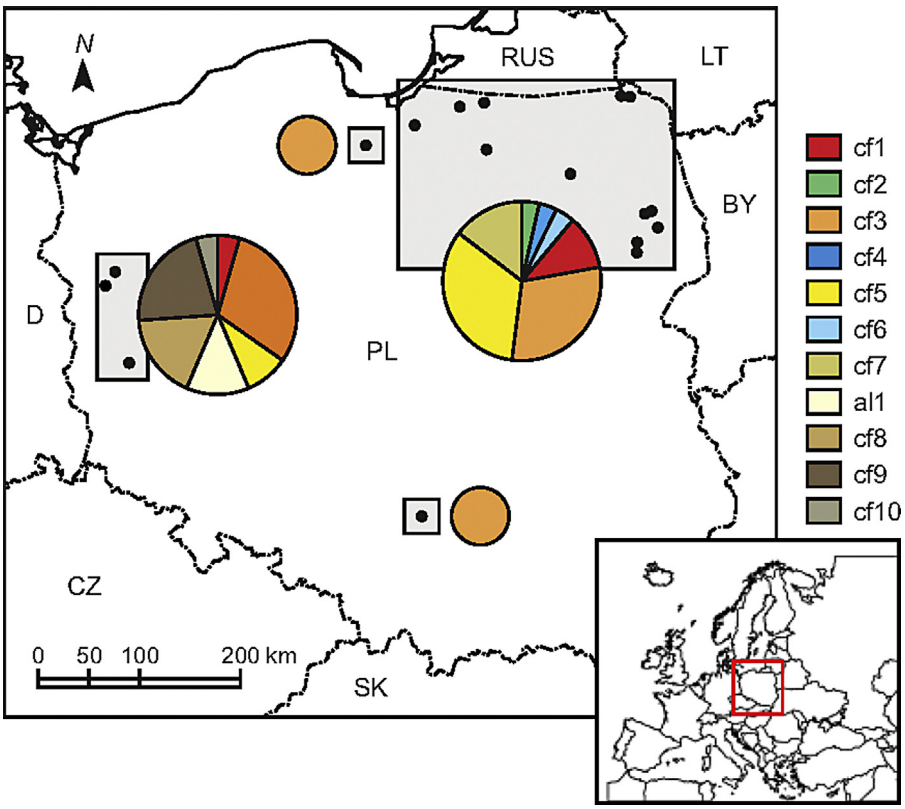

Fig. 1. Map of the study area of the Eurasian beavers and the distribution and frequency of detected 11 control region mtDNA haplotypes as a pie charts. Forest inspectorates where samples were collected indicated with dots. Haplotypes al1, Cf1, Cf3, Cf4, Cf5, Cf8, Cf9, and Cf10 belong to the western mtDNA lineage, while haplotypes Cf2, Cf6, and Cf7 belong to the eastern mtDNA lineage according to Durka et al. (2005), see also Fig. 5.

following 10 YCATS (Y chromosome conserved anchored tagged sequences) were amplified using universal primers (Hellborg and Ellegren 2003): DBY1, DBY3, DBY3/4, DBY4, DBY7, DBY8, DBY9, DBY14, UBE1Y6 and UTY11 in 44 *C. fiber* males from Poland. PCR reactions were performed with a GeneAmp PCR System 9700 (Applied Biosystems) in 5 μL volumes containing 2 μL genomic DNA (~20 ng), 1.7 μL Qiagen Multiplex PCR Master Mix (1×), 0.3 μL mix of primers (0.2 μM of each primer) and 1 μL RNase-free water. The reaction conditions were as follows: 15 min at 95 °C followed by 35 cycles with denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s and the final elongation for 30 min at 60 °C.

All sequencing reactions in both directions (for mtDNA and YCATS) were performed using the BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's protocol. The detection of sequencing reaction products was carried out on an ABI PRISM 3130xl or ABI 3130 Genetic Analysers (Applied Biosystems). Sequences were checked manually and aligned with use of the ClustalX algorithm in Bioedit 7.0.5.3 (Hall 1999).

Population genetic analyses, microsatellites

Microsatellite genotype frequencies were tested for deviations from Hardy–Weinberg expectations with exact tests of Guo and Thompson (1992) in Genepop 4 (Rousset 2008). The following genetic diversity parameters were estimated for each locus using FSTAT 2.9.3 (Goudet 1995): number of alleles (N_A), observed and expected heterozygosity (H_O and H_E) and F_{IS}. Tests of linkage disequilibrium between all pairs of loci were performed in Genepop using 10,000 permutations. Associated probability values were corrected for multiple comparisons using the Bonferroni adjustment for a significance level of 0.05.

To assess the autosomal genetic structuring of beavers over the entire sampled area, we used the Bayesian model-based clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000). We used the admixture model and correlated allele frequencies with no prior information about sample origin (Falush et al. 2003). We performed 10 independent runs for each K value from $K = 1$ to $K = 5$. Each run had 100,000 iterations following a burn-in of 100,000 iterations. To determine the most likely number of clusters, we used the lowest value of mean ln$P$ ($X|K$). Because the log-likelihood estimated by STRUCTURE often displays higher variance between runs for higher K values, we also calculated the rate of change in the log probability of data between successive K values (ΔK, Evanno et al. 2005) with use of STRUCTURE HARVESTER software (Earl and von Holdt 2012). For each individual, the fraction of its genotype derived from each genetic cluster was calculated using CLUMPP (Jakobsson and Rosenberg 2007) as the average across runs. Individuals that did not reach 0.7 of their genotype in any cluster were classified as of mixed ancestry or immigrants. This threshold was used to give an optimal balance between the efficiency and accuracy for categorizing individuals as admixed and non-admixed (Vähä and Primmer 2006).

To detect first generation migrants we used Bayesian assignment of Rannala and Mountain (1997) implemented in GeneClass 2.0. Probabilities of being a resident were derived for 10,000 simulated individuals and the cut-off criterion was set to $p < 0.005$. To compare the level of differentiation between these populations we computed pairwise F_{ST} using FSTAT 2.9.3.

Additionally, we used multilocus microsatellite data to perform factorial correspondence analysis (FCA) implemented in Genetix 4.05 (Dawson and Belkhir 2001) to explore the distribution of genetic variation graphically. FCA finds the uncorrelated linear combination of variables (allele frequencies at different loci) which explain the highest amount of variation contained in the data.

Measurements of genetic diversity were estimated for all genetically distinct groups as defined by STRUCTURE analysis. Mean number of alleles (N_A, as the number of individuals in each group was equal), observed and expected heterozygosity (H_O and H_E) and F_{IS} were estimated using FSTAT 2.9.3. The program BOTTLENECK 1.2.02 (Piry et al. 1999) was used to test for a recent bottleneck or expansion in each STRUCTURE defined population. An excess of observed gene diversity relative to the expected gene diversity for the number of alleles detected in the sample may indicate a population size reduction. Conversely, a deficit in the observed gene diversity may indicate that the population is growing (Piry et al. 1999). Two mutation models, considered appropriate for microsatellites, were applied: the strict Stepwise Mutation Model (SMM) and the Two-Phase Model (TPM). For the TPM, a model that includes both 90% single-step mutations and 10% multiple step mutations was used. Significant deviations in observed heterozygosity over all loci were tested using a non parametric Wilcoxon test.

Population genetic analyses, mitochondrial DNA

DnaSP 5.0 (Librado and Rozas 2009) was used to determine the number of haplotypes (h) and variable sites (S), and to calculate haplotype diversity (Hd) and nucleotide diversity (π). We also calculated pairwise F_{ST} and haplotype diversity measures (h, S, Hd and π) for STRUCTURE defined genetic groups. Relationships among the CR–mtDNA haplotypes were assessed using parsimony and distance methods. Haplotypes obtained in this study were analyzed along with haplotypes reported in Durka et al. (2005), covering the whole Eurasian range of *Castor fiber* as well as three haplotypes of western lineage (JF264886–JF264888) from Germany (Horn et al. 2010). All analyses were performed with PAUP* 4.0b10 (Swofford 2003). In maximum-parsimony (MP) analysis, gaps were treated as a fifth character to account for the insertions/deletions (indels) present in this noncoding region. MP searches were conducted by the branch and bound method. A neighbor joining (NJ) tree was constructed from the matrix of pairwise p distances. Trees were rooted with three *Castor canadensis* sequences (GenBank accession numbers AY623644–AY623646). Robustness of both MP and NJ trees was tested with 1000 bootstrap replicates. To explore the haplotype genealogies for mtDNA, median joining network was constructed in NETWORK v4.6.1.0 (http://www.fluxus-engineering.com). To check whether CR mtDNA sequences were noted previously in the literature, we compared our data with both, extant and ancient complete sequences of *Castor fiber* obtained from GenBank (Durka et al. 2005; Horn et al. 2010, 2014).

The frequencies of mtDNA haplotypes detected in this study are presented on the map of the sampling area as pie charts (Fig. 1).

Results

We obtained full microsatellite genotypes for 77 individuals (38 from NE Poland, 38 from W Poland and 1 sample from Pomerania). We were not able to genotype samples from museum specimens from the Silesia region, consequently they were excluded from microsatellite analysis. Three loci (*Cca9*, *Cca10* and *Cca15*) were monomorphic and they were excluded from further analysis. We detected 2–10 alleles per polymorphic locus (Table 1). All loci were in Hardy–Weinberg equilibrium when all samples were included, indicating that overall population structuring is limited. No significant linkage disequilibrium was detected between any pair of polymorphic loci.

According to the STRUCTURE analysis, microsatellite data clearly support the presence of two genetic clusters as both the probability of the data and ΔK (Evanno et al. 2005) were highest for $K = 2$ (Fig. 2). These two clusters correspond well to populations from W and NE Poland, indicating geographic structuring of microsatellite variation. Most individuals sampled in these respective regions had an overwhelming majority of their ancestry classified to the cluster characteristic for that region. The analysis classified 12 individuals from W and for 6 individuals from NE Poland as of admixed ancestry or immigrants (Fig. 3). Admixed individuals from W Poland had from 0.038 to 0.675 of their genome derived from the eastern cluster, whereas those from NE Poland had from 0.346 to 0.687 of their genome derived from the western cluster. MtDNA sequences were available for 6 of 12 admixed W Poland beavers, all of them had the western mtDNA type (Cf3 and Cf5). Among six admixed beavers from NE Poland five had the western (Cf1, Cf3, Cf5) and one the eastern mtDNA haplotype (Cf6). One individual sampled at a relatively long distance north from W Poland, in Pomerania (Fig. 1.) was classified to the NE cluster (0.99 of eastern ancestry), but had the western mtDNA type (Cf3). Among W Poland individuals with admixed ancestry there were first generation migrants detected by Geneclass2.0. Mitochondrial haplotypes for those individuals were not known. Similarly, in NE Poland three individuals were classified as first generation migrants. Two of those individuals had western mtDNA haplotypes and in one we detected an eastern mtDNA haplotype.

FCA confirmed the results of the STRUCTURE analysis: individuals from W and NE Poland separated along the first axis (Fig. 4.). While most samples cluster within a range of their respective group members, several samples are scattered widely along the second FCA axis. Although the separation of individuals from W and NE Poland is clearly visible, a number of admixed individuals are apparent. Similarly to STRUCTURE analysis, the individual from the Pomeranian region clustered together with beavers from NE Poland. Genetic differentiation between W and NE Poland was also confirmed by F_{ST} value of 0.076, being moderate and significantly different from zero ($p < 0.001$).

Measures of microsatellite variation in W and NE Poland are given in Table 2. The mean number of alleles per locus, F_{IS} and heterozygosities were similar for both groups, although we detected a slightly higher mean number of alleles in NE Poland. We did not detect any signature of heterozygote excess or deficit using the nonparametric Wilcoxon test in Bottleneck.

Among 65 sequenced individuals, 11 mtDNA CR haplotypes were identified (Fig. 1.). Only two of these haplotypes were reported earlier by Durka et al. (2005) (haplotype al1, GenBank accession number: DQ088700) and Horn et al. (2010) (GenBank accession number: JF264887, identical with our haplotype Cf5). The haplotypes obtained in our study were deposited in GenBank

Table 1
Characterization of microsatellite loci genotyped in 77 beaver individuals from Poland.

Locus	N_A	H_E	H_O	F_{IS}	p
Cca4	6	0.6183	0.5584	0.103	0.444
Cca5	3	0.3984	0.3611	0.101	1.000
Cca8	10	0.6641	0.6274	0.102	0.071
Cca13	4	0.6319	0.5974	0.061	0.398
Cca18	2	0.4190	0.3896	0.077	0.525

N_A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, fixation index; p, the probability of Hardy–Weinberg deviations.

Table 2
Genetic diversity of STRUCTURE defined beaver populations.

	N	N_A	H_E	H_O	F_{IS}	p
W	38	3.6	0.514	0.528	−0.028	0.423
NE	38	4.4	0.530	0.478	0.181	0.498

N_A, mean number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, fixation index; p, the probability of Hardy–Weinberg deviations.

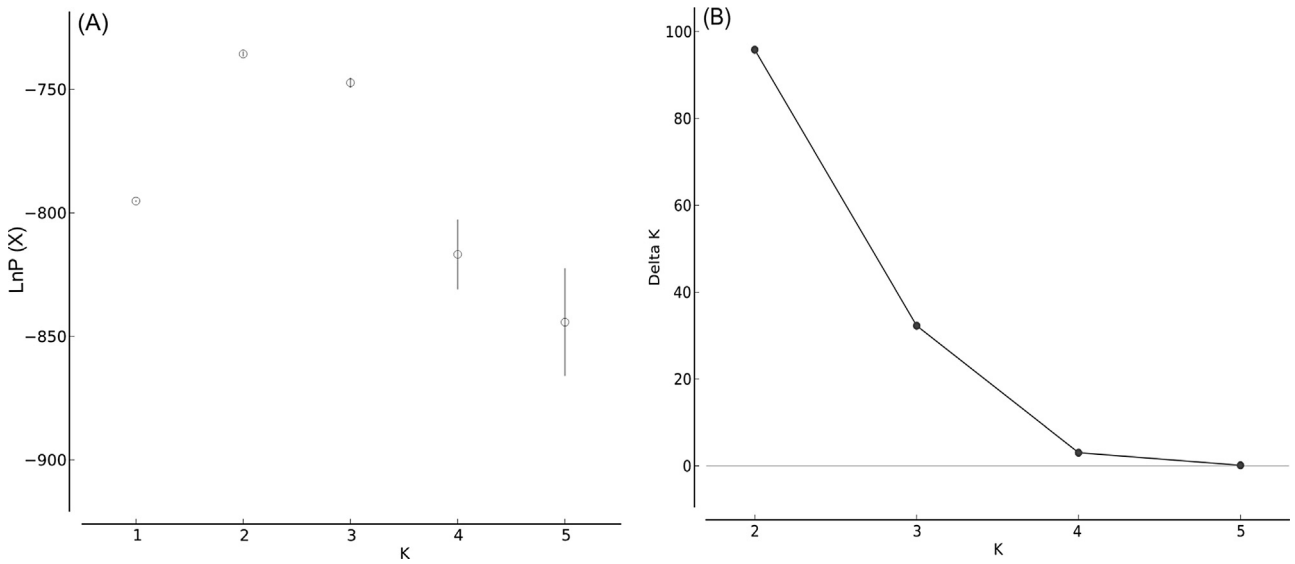

Fig. 2. (A) Mean ln$P$ (K) and SD of 10 replicates of STRUCTURE runs for each K performed on multilocus microsatellite data, where $K = 2$ is indicated as the best fit. Each run had 100,000 iterations following a burn-in of 100,000 iterations. (B) Delta K (ΔK) of Evanno et al. (2005), where $K = 2$ is indicated as the best fit for different levels of structuring. These two clusters correspond well to populations from Western and North-eastern Poland, indicating geographic structuring of microsatellite variation.

(accession numbers: KC693753–KC693762). The number of segregating sites (S) was 32 and 19 of these sites were parsimony informative. Haplotype diversity (Hd) was 0.769 and nucleotide diversity (π) was 0.0105. For population analysis haplotypes obtained in Pomerania and Silesia were excluded due to low sample size (1 and 3, respectively). Mitochondrial control region diversity revealed moderate and significant differentiation between W and NE populations ($F_{ST} = 0.13$; $p < 0.001$). In NE Poland we detected 7 haplotypes characterized by 28 segregating sites, Hd was 0.761 and π was 0.015. In W Poland we found also 7 haplotypes characterized by 11 segregating sites. Hd was 0.745 and π was 0.0045. Maximum parsimony and neighbour-joining analyses performed jointly for CR haplotypes detected in this study and those reported from the Eurasian range of the beaver in Durka et al. (2005) and Horn et al. (2010) demonstrated that the new haplotypes could be unambiguously classified into the eastern and western lineage (Fig. 5.). In NE Poland we detected haplotypes Cf2, Cf6 and Cf7 from the eastern mtDNA lineage but also haplotypes Cf1, Cf3, Cf4 and Cf5 from the western lineage. All beavers sampled in W Poland had control region mtDNA haplotypes (al1, Cf3, Cf8, Cf9 and Cf10) from

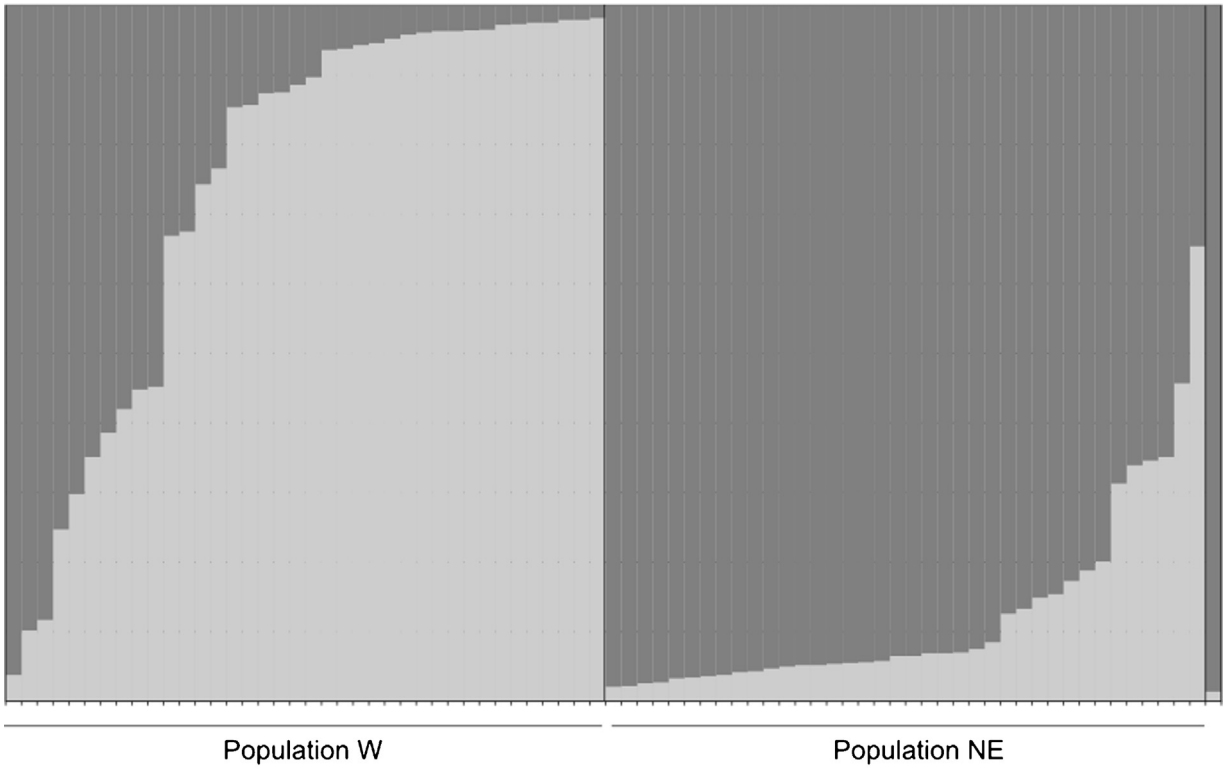

Fig. 3. Average individual membership in genetic groups assigned by STRUCTURE in comparison to sampling location (beavers populations from Western and North-eastern Poland, and from Pomerania region), for $K = 2$. The last bar represents an individual sampled in Pomerania region. Individuals sorted according to highest membership.

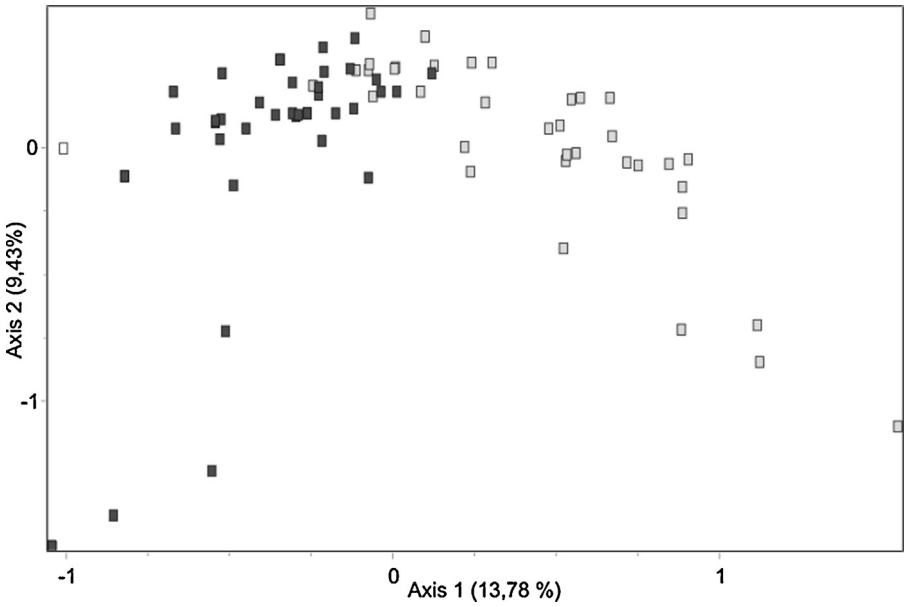

Fig. 4. Factorial Correspondence Analysis (FCA) highlighting individual clustering for the first two dimensions of variance performed on multilocus microsatellite data. The Eurasian beavers individuals sampled from populations in North-eastern Poland marked with dark grey, individuals sampled from populations in Western Poland marked with light grey, one individual sampled in Pomerania marked with white.

the western lineage. The most common haplotype was Cf3, found in both regions. Also in three samples from Silesia and one sample from Pomerania Cf3 haplotype was detected. The distribution of the haplotypes is presented in Fig. 1. The mtDNA network (Fig. 6.) also delineated two separate lineages within the Eurasian beaver, suggesting the presence of a strong phylogeographic structure. These lineages were separated by at least 10 mutation steps. The western lineage is characterized by more or less star-like shape with

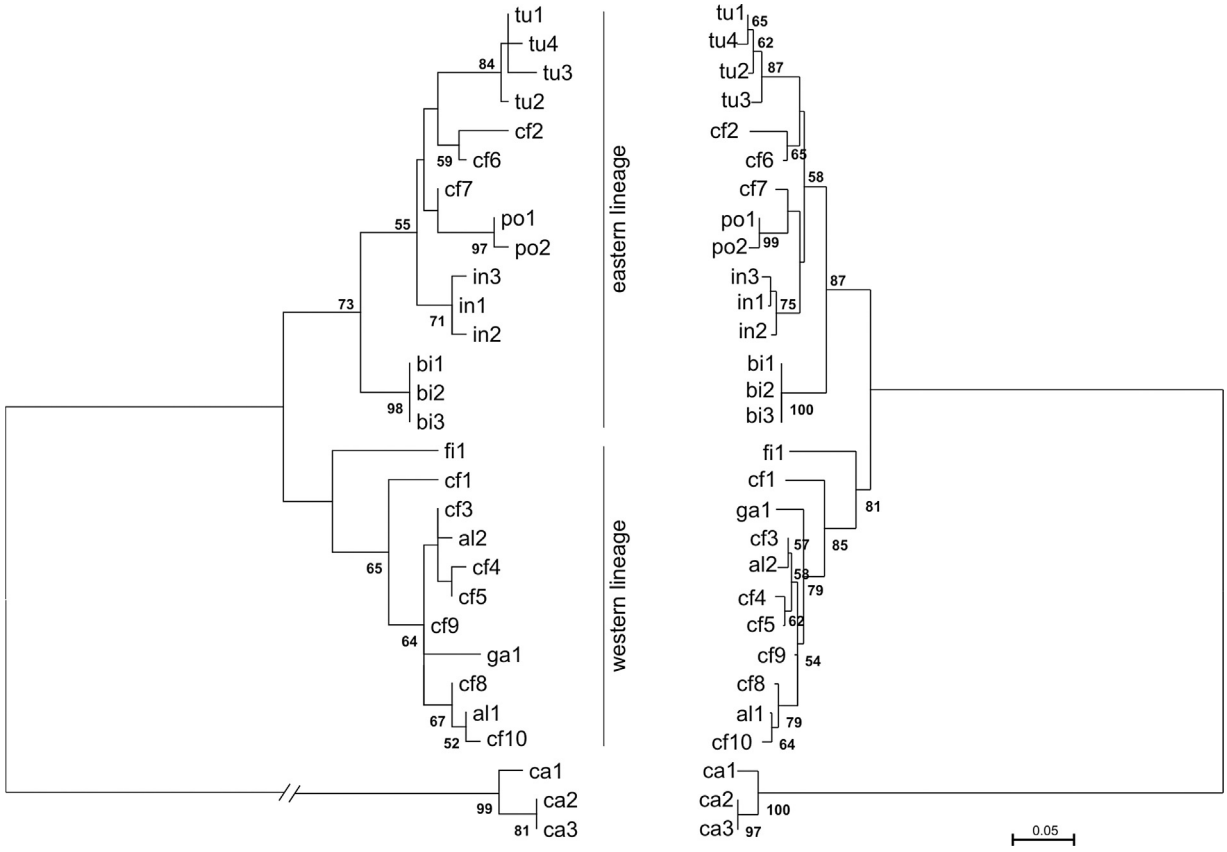

Fig. 5. The neighbor-joining (NJ; left) and the maximum parsimony (MP; right) tree constructed from *Castor fiber* control region mtDNA haplotypes (490 bp) obtained by Durka et al. (2005) and in this study (Cf1–Cf10). The NJ tree was constructed from the matrix of pairwise p distances, while the MP by using the branch and bound method. Both trees rooted with *Castor canadensis* haplotypes (ca1–ca3; GenBank accession numbers AY623644–AY623646). Numbers listed at nodes represent percent support for that node from 1000 bootstrap replicates.

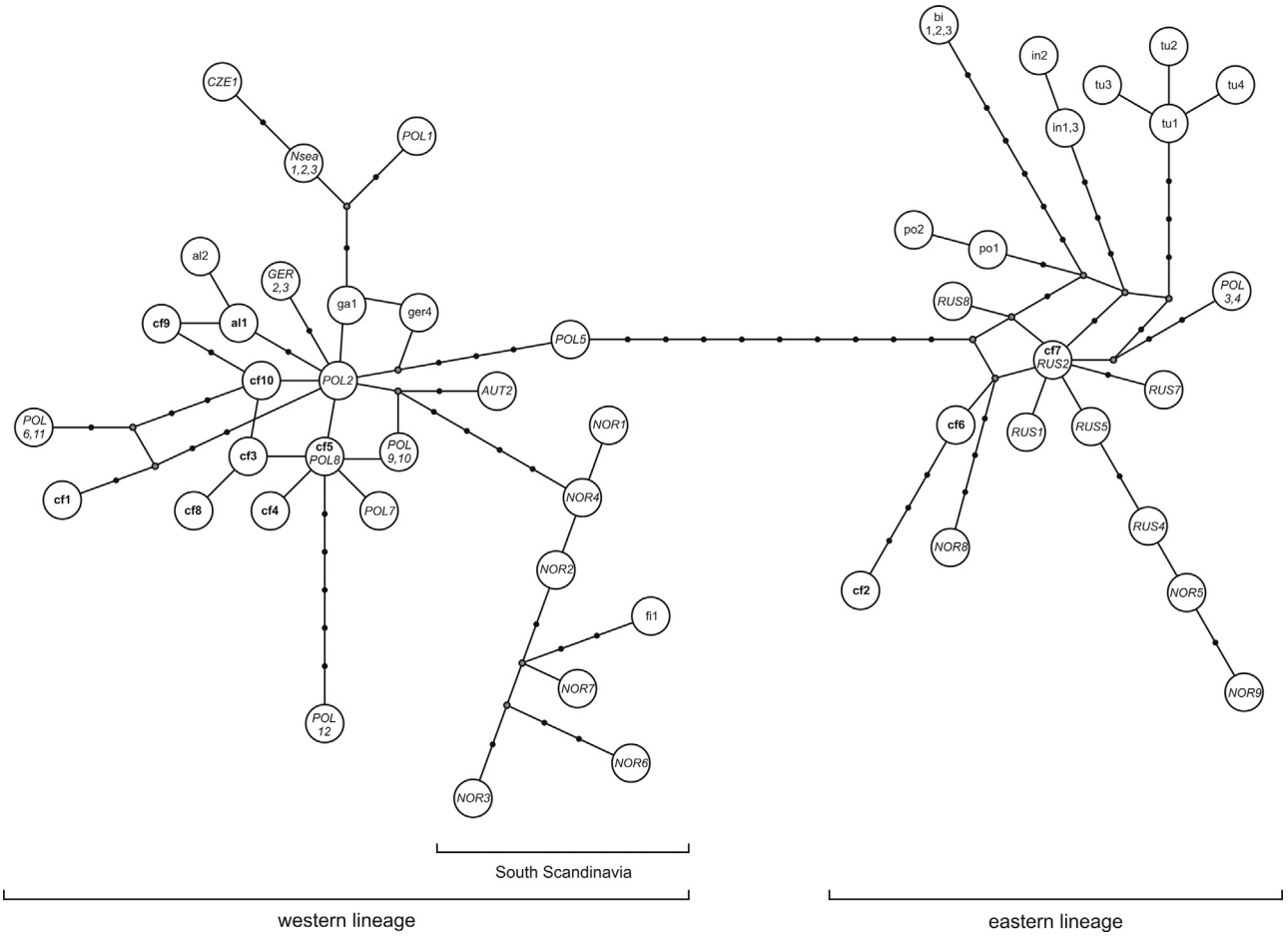

Fig. 6. Median-joining network showing relationships among Eurasian beavers mitochondrial DNA control region haplotypes (480 bp) belonging to the western and eastern lineages. Eleven mtDNA–CR haplotypes found in our study have codes from cf1 to cf10, the al1 haplotype was previously described by Durka et al. (2005). All haplotypes found in Poland (this study) are in bold. We also used ancient haplotypes from Horn et al. (2014) (shown in italics). The extant haplotypes: al, bi, fi, fin, ga, in, po, tu are from Durka et al. (2005), and ger4 (GenBank accession no.: JF264886) is from Horn et al. (2010). Missing haplotypes are shown as a grey dot.

South Scandinavian subclade. Interestingly, the ancient POL2 haplotype has central position at the network and it differs by a single substitution from extant Cf10 haplotype detected in our study. Moreover, ancient POL8 haplotype is identical with extant Cf5 haplotype that we found in Poland and Germany. This haplotype is ancestral with respect to extant Cf3, 4, 8 as well as ancient POL7, POL9 and POL10 haplotypes (Fig. 6.). The eastern mtDNA lineage is reticulated and contains several subclades with distinct haplotypes. Again, in Poland, we found Cf7 haplotype that was identical with the ancient RUS2 haplotype and two other extant haplotypes (Cf6 and Cf2) that differed from this haplotype by two and six mutations, respectively.

We successfully amplified and sequenced 6 out of 10 YCATS: DBY4 (236 bp), DBY7 (208 bp), DBY8 (134 bp), DBY9 (479 bp), DBY14 (230 bp), and UTY11 (263 bp), giving a total of 1550 bp of Y chromosome screened for variation. The DBY7 was the only one containing any variable positions, in this case C/T transversion: T was present in *C. f. birulai* (accession no: KF802798) and C in all other examined individuals (accession no: KF802797). At the species level, Hd for DBY7 was 0.053 and π was 0.00003. No polymorphism for the studied YCATS was found in beaver males from Poland.

Discussion

In this study we used mitochondrial and nuclear microsatellite as well as Y-linked markers in order to identify the patterns of genetic differentiation among beaver populations in Poland. We compared our mtDNA results with recently published ancient sequences of the species (Horn et al. 2014). It is apparent that a strong historic bottleneck followed by extensive management practices caused extreme fluctuations in population sizes and admixture between NE and W populations. These are the first results reporting the effects of multiple translocations and reintroductions on genetic structure of this species. We detected several new mtDNA haplotypes as well as significant genetic differentiation between the populations from W and NE Poland both in mtDNA and microsatellites. Substantial differentiation was not expected, as both populations were thought to be derived from the same mixed stock of eastern European origin. The diversity of the species was shaped by unexpected expansion from the west, the possible presence of relict population(s) as well as multiple translocations of insufficiently documented history.

Microsatellite differentiation

Our data on microsatellite diversity of beaver populations in Poland are the first enabling high-resolution assessment of population structure after restoration. There are currently no comparative nuclear data from other Eurasian beaver populations. Microsatellite diversity is moderate in comparison with values usually obtained for mammal species, and higher than that reported for severely bottlenecked populations (Frankham et al. 2002). This result does not confirm the expectation of extreme reduction of beaver genetic

diversity (Ellegren et al. 1993; Babik et al. 2005). A study of the North American beaver (*Castor canadensis*) in Illinois where the species was also reintroduced after population collapse in the 19th century revealed similar levels of genetic diversity (Crawford et al. 2009). Out of five polymorphic microsatellites used in our study, three were also used to examine genetic diversity in Illinois populations. Although we cannot directly compare genetic diversity of those two species, the number of alleles and expected heterozygosities in Polish populations were comparable or even exceeding those for Illinois populations (Crawford et al. 2009). We do not know whether microsatellite variation detected in the current study is higher or lower than in relict, post-bottleneck populations of the Eurasian beaver. One may suspect that variation is higher than in relict populations because the present-day Polish populations are derived from several source populations, which are known to be differentiated in mtDNA and MHC, but show very little intrapopulation variation (Babik et al. 2005; Ducroz et al. 2005; Durka et al. 2005). The recent availability of a large number of microsatellite (Pelz-Serrano et al. 2009; Frosch et al. 2012) and SNP (Senn et al. 2013) markers opens exciting opportunities to track the origin and history of translocations in the beaver. Polish populations will be an excellent object of such studies, especially if broad comparative data from all relict populations become available.

Microsatellite differentiation expressed as pairwise F_{ST} demonstrated moderate and significant differentiation between W and NE Poland, which does not support the reintroduction from a single stock of mixed origin. The genetic structuring observed between populations of the previously mentioned North American beaver populations revealed almost identical levels of population differentiation (F_{ST} = 0.068 versus 0.076 in our study, both values being significantly different from zero) (Crawford et al. 2009). This result can be explained by limited dispersal between populations despite relatively recent reintroductions acting in a similar way in populations of the North American beaver and in the Polish populations of the Eurasian beaver. According to the STRUCTURE analysis, the majority of individuals were assigned to the eastern or western clusters, broadly in agreement with positions of their sampling locations, although migrants and admixed individuals were also identified in each region. Despite the equal sample sizes, twice as many migrants or admixed individuals were detected in the W compared to NE Poland. This suggests extensive exchange of beavers between NE and W Poland, likely the result of numerous translocations. Interestingly, all individuals with admixed ancestry detected in Western Poland for which mtDNA was assayed were of the western mitochondrial type. Taken together, these results indicate that beavers from the western mtDNA lineage have contributed considerably to the Polish population, particularly in W Poland. Most of this contribution appears to result from natural immigration of the beavers from Germany, but we cannot exclude translocations of German beavers to eastern Poland during World War II; the evidence for such translocations is however equivocal (Halley and Rosell 2002). A recent model of beaver population dispersal based on the data of beaver expansion in Czech Republic predicted substantially higher spread rate along watercourses (15–20 km per year) than classical models, and a progressive space filling rather than increasing population density as a result beaver population growth (Barták et al. 2013). Currently the Eurasian beaver occurs over the entire territory of Poland (http://www.iop.krakow.pl/ssaki/Gatunek.aspx?spID=61) and the number of detected migrants would probably be even higher if the sampling would be more extensive.

We did not detect signatures of population bottleneck in any of the regions. Taking into account that the species survived only in few refugia across its range that resulted in reduced genetic diversity (Ellegren et al. 1993; Babik et al. 2005; Durka et al. 2005) and Polish beaver populations were established with a limited number of individuals only about 60 years ago the effects of size reduction was expected. The lack of bottleneck effect due to population size fluctuations could be due to two factors. First, the power of five microsatellite loci to detect a signature of a bottleneck is low. Second, Polish beaver population was established from individuals originating from several eastern relict populations, in effect, beavers in recolonized area show moderate or even high diversity. Migration from the German populations that represent western mtDNA lineage followed by admixture could further erase the signal of the bottleneck.

Haplotype diversity in Polish beaver populations (mtDNA and YCATS)

The interpretation of mtDNA variation detected in the present study is greatly facilitated by previously published data. According to these studies, the extreme bottleneck that affected all Eurasian beaver populations resulted in a significant reduction of intrapopulation diversity and strong population structure in both neutral mtDNA (Durka et al. 2005; Horn et al. 2014) and effectively selected (MHC) markers (Babik et al., 2005). Durka et al. (2005) found extreme genetic structuring within the present range of *C. fiber*, with strong phylogeographical division between Western populations from Germany, France, and Norway and remaining populations located further to the east. This pattern was explained by strong reduction in gene flow accompanied by beaver extirpation throughout its range or by postglacial expansion followed by elimination of a part of neighboring populations. Although this result may hold in previously studied populations, our results show that Polish beaver populations exhibit more complex genetic structure. In our study we obtained mitochondrial haplotypes for 65 beavers sampled over a relatively small area. Among those samples we found 11 haplotypes that group within either eastern (4 haplotypes) or western (8 haplotypes) mtDNA haplogroups detected by Durka et al. (2005), 9 of them not being reported previously. Interestingly, our study, together with Durka et al. (2005) and Horn et al. (2010) revealed similar number of haplotypes for western and eastern mtDNA lineages: 12 and 15, respectively. This clearly indicates that either relict populations sampled by Durka et al. (2005), with 7–39 individuals sequenced, are themselves genetically structured and previous sampling detected only a fraction of variation present there, or additional, previously unknown beaver populations survived the historical bottleneck. It seems likely that such populations could have also survived in Belarus, Ukraine and western Russia as well as even in Poland. Published maps (Nolet and Rosell, 1998; Rosell et al. 2012) show multiple relict areas e.g. in the Pripet marshes. Remarkably, several new mtDNA haplotypes found in Poland (this study) were identical or very similar to the ancient haplotypes from Poland and Russia (Horn et al. 2014). This indicates that beavers could have not been extinct during the WW II in Poland. Moreover, ancient POL2 and POL8 haplotypes that belong to the western lineage are ancestral to some of extant haplotypes in Poland. Thus, it seems very probable that at least some extant beavers are descendants of ancient populations that lived in Central Europe (e.g. Eastern Germany, Poland) several thousand years ago. More extensive, carefully designed sampling of the known relict areas will be needed to distinguish between the possibilities outlined above.

Our results show that western lineage haplotypes are common and widespread in Polish beaver populations. This pattern can be explained, similarly as in case of microsatellite differentiation, by extensive migration of beavers from the western bank of the Oder river (acting as a migratory corridor) as a result of strong beaver range expansion in Germany (Kautenburger and Sander 2008). Since many rivers join the Oder river on the Polish side, this immigration from Germany followed by eastwards expansion

could be considerably facilitated. If this has been the case, beaver populations in Eastern Germany and Western Poland would be genetically very close. This is quite probable as al1 and Cf5 mtDNA haplotypes were found both, in Poland (this study) and Germany (Durka et al. 2005; Horn et al. 2010). The presence of only western lineage mtDNA haplotypes in Western Poland is striking in the light of numerous translocations from Eastern Poland over last 60 years and the detection of migrants from the NE population in the Western region. This would suggest that the contribution of beavers migrating from the western side of Oder river (Germany) to W Poland has been higher than that of beaver translocated from northeastern Poland. The most frequent haplotype found in this study (Cf3) belongs to the western haplogroup and is present in high frequencies both in W and NE Poland. This can be explained either by extensive migration of beavers from Germany possessing mtDNA haplotypes of western lineage as suggested above or possibly by poorly documented translocations from this country during the WW II to Poland. Alternatively, the presence of western lineage in eastern European countries (e.g. Belarus and possibly Western Russia) acting as a source populations for reintroductions in Poland could have resulted in the predominance of western lineage in Poland. This is probably the case, as haplotypes of western lineage were found in Belarus, especially in the Voronezh region (with 100% frequency) as well as in Kirov Oblast (Russia, with frequency of 81.3%; Senn et al. 2014). The presence of western mtDNA lineages in Eastern Poland was also documented for the weasels, *Mustela nivalis* (McDevitt et al. 2012).

The presence of a single Y chromosome haplotype among Polish males of *C. fiber* from the western and eastern mtDNA clades was not unexpected. Generally, low levels of Y polymorphism have been recorded in species of different mammalian natural populations, with Y chromosome sequences being completely monomorphic in lynx and reindeer (Hellborg and Ellegren 2004). On the other hand, the different Y-chromosome haplotype present in *C. fiber bilurai* may support its subspecific status.

Conservation implications

The spectacular beaver recovery in many European countries including Poland, which has resulted in its occurrence nearly all over the country, is one of rare examples of a successful translocation program followed by natural expansion. The beaver is presently one of the most widely distributed medium-size mammals in Poland. Beavers cause considerable damage to agriculture and forestry which is the main reason behind increasing social conflict concerning the species. As the beaver is a protected species, damages are compensated by the state; however the number and total compensation value paid have dramatically increased. The only means of population regulation are destruction of dams, and rare provisional shooting under detailed supervision of nature conservation authorities. Therefore the establishment of a comprehensive national management plan of the beaver populations is urgently needed.

According to Durka et al. (2005), western and eastern mitochondrial lineages that constitute reciprocally monophyletic units should be treated as separate evolutionarily significant units (ESUs, *sensu* Moritz 1994), and in consequence the management of beaver populations belonging to western and eastern subspecies should be performed separately as they constitute distinct conservation units. The main recommendation was of using western subspecies for reintroductions in western and central Europe, while using eastern subspecies east of the Oder River. Our results show that mixing of populations that belong to eastern and western mtDNA lineages in Poland has already occurred as a result of both natural expansion and human translocations. Interestingly, the position of a contact zone between the lineages is very similar to this in ancient times (Horn et al. 2014). There are reports of relatively poor performance of beavers derived from non-admixed populations, which may be a sign of inbreeding depression (reviewed in Halley 2011). On the other hand, high viability and the rapid range and demographic expansion of beaver in Poland and in many European countries do not provide any evidence of outbreeding depression (Halley 2011). Therefore is seems that a realistic recommendation would be to limit translocations in the near future as natural immigration from Germany may have played a more significant role than previously assumed and monitor closely beaver populations with use of genetic markers.

Acknowledgements

This work was supported from the National Science Center project grant number N304 098335 and by the Institute of Nature Conservation PAS and BST-102 (University of Bialystok) to M. Świsłocka. We thank Walter Durka for providing samples of beaver subspecies, Jan Goździewski and Polish forestry authorities for providing samples from Polish populations.

References

Abbot, R., Albach, D., Ansell, S., et al., 2013. Hybridization and speciation. J. Evol. Biol. 26, 229–246.
Avise, J.C., 2004. Phylogeography. The History and Formation of Species. Harvard University Press, Cambridge, MA.
Babik, W., Durka, W., Radwan, J., 2005. Sequence diversity of the MHC DRB gene in the Eurasian beaver (*Castor fiber*). Mol. Ecol. 14, 4249–4257.
Barták, A., Vorel, S., Šimová, P., Puš, V., 2013. Spatial spread of Eurasian beavers in river networks: a comparison of range expansion rates. J. Anim. Ecol. 82, 587–597.
Cheney, L.C., 1995. An Assessment of Genetic Variation Within and Between Sea Otter (*Enhydra lutris*) Populations of Alaska and California. Moss Landing Marine Laboratories, California State University, San Jose.
Crawford, J., Liu, Z., Nelson, T., Nielsen, C., Bloomquist, C., 2008. Isolation and characterization of microsatellite loci in the beaver (*Castor canadensis*). Mol. Ecol. Resour. 8, 616–618.
Crawford, J.C., Liu, Z., Nelson, T.A., Nielsen, C.K., Bloomquist, C.K., 2009. Genetic population structure within and between beaver (*Castor canadensis*) populations in Illinois. J. Mammal. 90, 373–379.
Currat, M., Ruedi, M., Petit, R.J., Excoffier, L., 2008. The hidden side of invasions: massive introgression by local genes. Evolution 62, 1908–1920.
Czech, A., 2010. Bóbr – budowniczy i inżynier. Fundacja Wspierania Inicjatyw Ekologicznych, Kraków.
Dawson, K.J., Belkhir, K., 2001. A Bayesian approach to the identification of panmictic populations and the assignment of individuals. Genet. Res. 78, 59–77.
Djoshkin, W.W., Safonow, W.G., 1972. Die Biber der Alten und Neuen Welt. Wittenberg, A. Ziemsen Verlag.
Ducroz, J.-F., Stubbe, M., Saveljev, A., Heidecke, D., Samjaa, R., Ulevičius, A., Stubbe, A., Durka, W., 2005. Genetic variation and population structure of the Eurasian beaver *Castor fiber* in Eastern Europe and Asia based on mtDNA sequences. J. Mammal. 86, 1059–1067.
Durka, W., Babik, W., Ducroz, J-F., Heidecke, D., Rosell, F., Samjaa, R., Saveljev, A.P., Stubbe, A., Ulevičius, A., Stubbe, M., 2005. Mitochondrial phylogeography of the Eurasian beaver *Castor fiber* L. Mol. Ecol. 14, 3843–3856.
Dzięciołowski, R., Goździewski, J., 1999. The reintroduction of the European beaver *Castor fiber* in Poland: a success story. In: Busher, P., Dzięciołowski, R. (Eds.), Beaver Protection, Management and Utilization in Europe and North America. Kluwer Academic/Plenum Publishers, New York, pp. 31–35.
Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Res. 4, 359–361.
Ellegren, H., Hartman, G., Johansson, M., Andersson, L., 1993. Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers. Proc. Natl. Acad. Sci. U.S.A. 98, 8150–8153.
Evanno, G., Regnaut, U.T., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Excoffier, L., Foll, M., Petit, R.J., 2009. Genetic consequences of range expansions. Annu. Rev. Ecol. Evol. Syst. 40, 481–501.
Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
Frahnert, S., 2000. Ontogenetic changes of cranial proportions in eaver, *Castor fiber* L., 1758 (Rodentia Castoridae): taxonomical mplications and functional aspects. Bonner Zoologische Beiträge 49, 131–153 [in German].

Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Conservation Genetics. Cambridge University Press, Cambridge.
Frosch, C., Haase, P., Nowak, C., 2012. First set of microsatellite markers for genetic characterization of the Eurasian beaver (*Castor fiber*) based on tissue and hair samples. Eur. J. Wildl. Res. 57, 679–682.
Goudet, J., 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. J. Hered. 86, 485–486.
Guo, S., Thompson, E.A., 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48, 361–372.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Halley, D.J., 2011. Sourcing Eurasian beaver *Castor fiber* stock for reintroductions in Great Britain and Western Europe. Mammal Rev. 41, 40–53.
Halley, D.J., Rosell, F., 2002. The beaver's reconquest of Eurasia: status, population development and management of a conservation success. Mammal Rev. 32, 153–178.
Hellborg, L., Ellegren, H., 2003. Y chromosome conserved anchored tagged sequences (YCATS) for the analysis of mammalian male-specific fragments. Mol. Ecol. 12, 283–291.
Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. R. Soc. Lond. B 359, 183–195.
Hofreiter, M., Serre, D., Rohland, N., Rabeder, G., Nagel, D., Conard, N., Münzel, S., Pääbo, S., 2004. Lack of phylogeography in European mammals before the last glaciation. Proc. Natl. Acad. Sci. U.S.A. 101, 12963–12968.
Hofreiter, M., Stewart, J., 2009. Ecological change, range fluctuations and population dynamics during the pleistocene. Curr. Biol. 19, R584–R594.
Horn, S., Teubner, J., Heidecke, D., 2010. Mitochondrial DNA of beavers (*Castor*) in Germany. Artenschutzreport 26, 72–78.
Horn, S., Durka, W., Wolf, R., Ermala, A., Stubbe, A., Stubbe, M., Hofreiter, M., 2011. Mitochondrial genomes reveal slow rates of molecular evolution and the timing of speciation in beavers (Castor), one of the largest rodent species. PLoS ONE 6.
Horn, S., Prost, S., Stiller, M., Makowiecki, D., Kuznetsova, T., Benecke, N., Pucher, E., Hufthammer, A.K., Schouwenburg, C., Shapiro, B., Hofreiter, M., 2014. Ancient mitochondrial DNA and the genetic history of Eurasian beaver (*Castor fiber*) in Europe. Mol. Ecol. 23, 1717–1729.
Jakobsson, M., Rosenberg, N., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806.
Kautenburger, R., Sander, A., 2008. Population genetic structure in natural and reintroduced beaver (*Castor fiber*) populations in Central Europe. Anim. Biodivers. Conserv. 31, 25–35.
Keller, S.R., Taylor, D.R., 2010. Genomic admixture increases fitness during a biological invasion. J. Evol. Biol. 23, 1720–1731.
Kolbe, J.J., Larson, A., Losos, J.B., De Queiroz, K., 2008. Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. Biol. Lett. 4, 434–437.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
McDevitt, A.D., Zub, K., Kawałko, A., Oliver, M.K., Herman, J.S., Wójcik, J.M., 2012. Climate and refugial origin influence the mitochondrial lineage distribution of weasels (*Mustela nivalis*) in a phylogeographic suture zone. Biol. J. Linn. Soc. 106, 57–69.
Moritz, C., 1994. Defining 'evolutionary significant units' for conservation. Trends Ecol. Evol. 9, 373–375.
Nolet, B.A., Rossel, F., 1998. Comeback of the beaver *Castor fiber*: an overview of old and new conservation problems. Biol. Conserv. 83, 165–173.
Pelz-Serrano, K., Munguia-Vega, A., Piaggio, A.J., Neubaum, M., Munclinger, P., Pártl, A., van Riber, C., Culver, M., 2009. Development of nine new microsatellite loci for the American beaver, *Castor canadensis* (Rodentia: Castoridae), and cross-species amplification in the European beaver, *Castor fiber*. Mol. Ecol. Resour. 9, 551–554.
Petit, R.J., Excoffier, L., 2009. Gene flow and species delimitation. Trends Ecol. Evol. 24, 386–393.
Piry, S., Luikart, G., Cornuet, J-M., 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J. Hered. 95, 536–539.
Pritchard, J.K., Stevens, M., Donnely, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. USA 94, 9197–9201.
Rosell, F., Campbell-Palmer, R.I., Parker, H., 2012. More genetic data are needed before populations are mixed: response to Sourcing Eurasian Beaver *Castor fiber* stock for reintroductions in Great Britain and Western Europe. Mammal Rev. 42, 319–324.
Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.
Saccone, C., Attimonelli, M., Sbisa, E., 1987. Structural elements highly preserved during the evolution of the D-loop-containing region in vertebrate mitochondrial-DNA. J. Mol. Evol. 26, 205–211.
Schwab, G., Lutschinger, G., 2001. The return of the beaver (*Castor fiber*) to the Danube watershed. In: Czech, A., Schwab, G. (Eds.), The European Beaver in a New Millennium. Carpathian Heritage Society, Cracow, Poland, pp. 47–50.
Senn, H., Ogden, R., Frosch, C., Syruckova, A., Campbell-Palmer, R., Munclinger, P., Durka, W., Kraus, R., Saveljev, A., Nowak, C., Stubbe, A., Stubbe, M., Michaux, J., Lavrov, V., Samiya, R., Ulevicius, A., Rosell, F., 2014. Nuclear and mitochondrial genetic structure in the Eurasian beaver (Castor fiber) – implications for future reintroductions. Evolutionary Applications. (in press).
Senn, H., Ogden, R., Cezard, T., Gharbi, K., Iqbal, Z., Johnson, E., Kamps-Hughes, N., Rosell, F., McErwing, R., 2013. Reference-free SNP discovery for the Eurasian beaver from restriction site-associated DNA paired-end data. Mol. Ecol. 22, 3141–3150.
Shafer, A.B.A., Cullingham, C.I., Côté, S.D., Coltman, D.W., 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. Mol. Ecol. 19, 4589–4621.
Swofford, D.L., 2003. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4. Sinauer Associates, Sunderland, MA.
Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7, 453–464.
Vähä, J.P., Primmer, C.R., 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Mol. Ecol. 15, 63–72.
Żurowski, W., 1979. Preliminary results of European beaver reintroduction in the tributary streams of the Vistula River. Acta Theriol. 24, 85–91.
Żurowski, W., 1980. Bóbr europejski w Polsce. Przegląd Hodowlany 11, 18–23.
Żurowski, W., 1992. *Castor fiber* (Linne, 1758) – Bóbr europejski. In: Głowaciński, Z. (Ed.), Polska czerwona księga zwierząt. PWRiL, Warszawa, pp. 56–59.