Population genetic structure in natural and reintroduced beaver (Castor fiber) populations in Central Europe

R. Kautenburger & A. C. Sander


Abstract
Population genetic structure in natural and reintroduced beaver (Castor fiber) populations in Central Europe.—Castor fiber Linnaeus, 1758 is the only indigenous species of the genus Castor in Europe and Asia. Due to extensive hunting until the beginning of the 20th century, the distribution of the formerly widespread Eurasian beaver was dramatically reduced. Only a few populations remained and these were in isolated locations, such as the region of the German Elbe River. The loss of genetic diversity in small or captive populations through genetic drift and inbreeding is a severe conservation problem. However, the reintroduction of beaver populations from several regions in Europe has shown high viability and populations today are growing fast. In the present study we analysed the population genetic structure of a natural and two reintroduced beaver populations in Germany and Austria. Furthermore, we studied the genetic differentiation between two beaver species, C. fiber and the American beaver (C. canadensis), using RAPD (Random Amplified Polymorphic DNA) as a genetic marker. The reintroduced beaver populations of different origins and the autochthonous population of the Elbe River showed a similar low genetic heterogeneity. There was an overall high genetic similarity in the species C. fiber, and no evidence was found for a clear subspecific structure in the populations studied.

Key words: Beaver, Castor fiber, Castor canadensis, Genetic diversity, RAPD, Reintroduction.

Resumen
Estructura genética en poblaciones naturales y reintroducidas de castor (Castor fiber) en Europa Central.—El castor euroasiático (Castor fiber Linnaeus, 1758) es la única especie autóctona del género Castor en Europa y Asia. Debido a la intensa presión cinegética a la que fue sometido hasta principios del siglo XX, su amplia distribución se vio drásticamente reducida. Tan sólo sobrevivieron algunas poblaciones en áreas aisladas, como por ejemplo en la zona del río Elba en Alemania. La pérdida de diversidad genética en poblaciones pequeñas o criadas en cautividad, causada por la deriva genética y la endogamia, supone un grave problema para la conservación de esta especie. Por otro lado, los ensayos de su reintroducción en distintas zonas de Europa han puesto de manifiesto que las poblaciones poseen una gran viabilidad y altas tasas de crecimiento. En el presente estudio se ha analizado la estructura genética de una población natural y dos reintroducidas en Alemania y Austria. Además, se muestra la diferenciación genética entre dos especies de castor, el castor euroasiático y el castor americano (C. canadensis), utilizando RAPD (polimorfismo de fragmentos de ADN amplificados al azar) como marcador genético. La población de castor reintroducida a partir de diferentes orígenes y las poblaciones autóctonas del río Elba muestran una baja heterogeneidad genética. Existe una alta semejanzá genética en la especie C. fiber, no hallándose evidencias de una estructura subspecífica en las poblaciones estudiadas.

Palabras clave: Castor, Castor fiber, Castor canadensis, Estructura genética, RAPD, Reintroducción.

(Received: 11 I 08; Conditional acceptance: 13 III 08; Final acceptance: 9 IX 08)


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Introduction

The genus Castor is the only living representative of the family Castoridae, with two species: Castor fiber, Linnaeus 1758 and Castor canadensis, Kuhl 1820 (Heidecke, 1998). C. fiber was once widespread in the holarctic, from Europe to northern Asia, as the result of glacial and postglacial climatic changes (Veron, 1992). However, populations in western Europe were limited to a few isolated sites (in the Elbe River in Germany, the Rhône River in France, and southern Norway) by the end of the 19th century due to over-hunting for pelts, meat and castoreum (a secretion from their scent glands). Habitat loss was also probably a contributory factor. A few small populations survived further east in Belarus, Russia and Mongolia. Only a local population of approximately 200 individuals survived in Germany, in the region of the Elbe River.

Since 1966 extensive reintroduction programs have reinstated beavers in many European countries. In western Germany, the first reintroduction program took place in the 1960s when beavers from Poland and Russia were released into the Danube watershed in Bavaria. In total, 120 beavers (C. fiber) of different origins were reintroduced along the rivers Danube and Inn between 1968 and 1975. By 2000, their numbers had increased to over 5000 individuals (Schwab & Lutschinger, 2001). Reintroduction programmes were also started in Austria and between 1976 and 1988 a total of 42 beavers, including 5 C. canadensis, were released along the Danube River east of Vienna. (Kollar, 1992). The population size in 2001 was estimated at approximately 1300 animals (Schwab & Lutschinger, 2001), but it is unknown how many of these were C. canadensis (Kollar & Seiter, 1990; Sieber, 1998). Nowadays, reintroduced populations in several regions in Europe are highly viable and growing fast, with beavers emigrating from Bavaria and Austria to adjacent areas (Halley & Rosell, 2002).

It is generally accepted that the local extinction of a species is followed by a bottleneck effect, implementing a reduction in the genetic diversity of the population (Avise, 1994). Reintroduction of a species with a few individuals leads to an artificial bottleneck, and this also reduces genetic diversity. The genetic impact of a potential bottleneck in the natural population and a founder effect in the reintroduced populations of C. fiber are still unclear. Nevertheless, successful reintroduction suggests that C. fiber is not sensitive to inbreeding and population growth does not appear to have been reduced due to any potential loss of genetic diversity (Nolet & Bavoco, 1996).

For conservation management strategies, it is necessary to analyse the genetic structure of natural and reintroduced beaver populations in order to ensure long-term viability of this key-species in riverine ecosystems, to preserve genetic diversity within the species and to prevent sensitivity to potential disease and parasitism (Nolet et al., 1997; Babik et al., 2005).

In the present study, we analysed the population genetic structure of two reintroduced beaver populations in the Danube River system east of Vienna (Austria) and in Bavaria (Germany) and one natural population of C. fiber in the Elbe River system in eastern Germany, using random amplified polymorphic DNA (RAPD) (Welsh & McClelland, 1990; Williams et al., 1990). Multilocus DNA markers are important for population studies, because they reveal many polymorphic loci distributed over the genome (Zhivotovsky, 1999; Krauss, 2000). Despite some methodological shortcomings (e.g. Bielawski et al., 1995; Grosberg et al., 1996), this fast and low-cost approach of RAPD–PCR has proven to deliver very useful information on population genetic structure and taxonomy without previous sequence information (e.g. Nebauer et al., 2000; Vucetic et al., 2001; Callejas & Ochando, 2002; Vandewoestijne & Baguette, 2002; Kautenburger, 2006a, 2006b).

This study addresses the following questions: Has the natural population of C. fiber in the Elbe River system undergone a loss in genetic diversity due to the severe bottleneck over the 19th century as compared to the reintroduced C. fiber populations from different origins in the Danube River system, and can RAPD markers reveal a genetic differentiation among the analysed beaver populations?

Material and methods

Study species

The genus Castor consists of two species: the Eurasian beaver C. fiber and the American beaver C. canadensis. The two species have a semi-aquatic life-style and are very similar in appearance and behaviour (Nolet, 1997). Beavers live in freshwater habitats lined by rich vegetation and they use trees to build dams and lodges (Macdonald et al., 2000). They are monogamous and live in small colonies, typically an adult pair and their offspring. They produce up to three young per year in a single litter (Wilsson, 1971). Beavers have an average life expectancy of seven to eight years. Dispersal usually takes place at a year and a half to two years of age and maximum distance recorded is about 170 km (Heidecke, 1984).

DNA samples

C. fiber is a protected animal species in Germany and Austria, as in many parts of Europe, and it is still under threat of extinction (IUCN red list category: NT. Near threatened; European Union red list category: LC. Least concern). Muscle tissue is therefore collected from dead animals (accident, or natural death). A total of 35 individuals were sampled for DNA typing as shown in table 1. Tissue samples were taken from 31 C. fiber and four C. canadensis. The C. fiber samples were collected from three different populations: 11 animals from the autochthonous Elbe River population (two that had been reintroduced into the Kinzig River in Hessen, Germany, and one that had been reintroduced into the Prims River in Saarland, Germany; 13 individuals from the reintroduced Bavarian Danube population;
and seven from the reintroduced Austrian Danube population east of Vienna (fig. 1) For comparison, four American beavers, three from Bavaria and one from Austria, were included as the out-group for the statistical analysis. All samples were stored at –20°C until further DNA analyses.

**Table 1. Overview of the analysed beaver samples:** Pop. Population; Ss. Sample size.

*Tabla 1. Muestras de castores analizadas: Pop. Población; Ss. Tamaño de la muestra.*

<table>
<thead>
<tr>
<th>Pop</th>
<th>Origin</th>
<th>Ss</th>
<th>Species/Subspecies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>Austria/Danube</td>
<td>7</td>
<td>Castor fiber spp.</td>
</tr>
<tr>
<td>Canada</td>
<td>Austria/Danube</td>
<td>1</td>
<td>Castor canadensis</td>
</tr>
<tr>
<td>Elbe</td>
<td>Germany/Kinzig–Prims</td>
<td>3</td>
<td>Castor fiber albicus</td>
</tr>
<tr>
<td>Elbe</td>
<td>Germany/Elbe</td>
<td>8</td>
<td>Castor fiber albicus</td>
</tr>
<tr>
<td>Bavaria</td>
<td>Germany/Danube</td>
<td>13</td>
<td>Castor fiber spp.</td>
</tr>
<tr>
<td>Canada</td>
<td>Germany/Danube</td>
<td>3</td>
<td>Castor canadensis</td>
</tr>
</tbody>
</table>

**DNA extraction**

Genomic DNA was extracted from muscle tissue using the salt–chloroform method adapted from Mullenbach et al. (1989). Frozen tissue (50–60 mg) was grounded in liquid nitrogen, transferred to a sterile
tube with 0.5 ml of extraction buffer (160 mM saccharose, 80 mM EDTA, 100 mM Tris/HCl, pH 8.0), and 20 µl proteinase K (20 mg/ml) and incubated for 16 hours overnight at 60°C. After the addition of 180 µl 6 M NaCl, proteins and lipids were removed using two extraction steps with 700 µl phenol–chloroform–isoamyl alcohol (25:24:1). DNA was precipitated by addition of twice the volume of ice–cold ethanol. The DNA pellet was recovered by centrifugation, washed in 70% ethanol, dried and dissolved in 500 µl H2O. DNA was quantified and qualified with a photometer (260 nm for the concentration, ratio of 260 nm and 280 nm for the purity); additionally, samples were checked on a 1.4% agarose gel.

RAPD reactions

Ten oligonucleotide (10mer) primers (Carl Roth GmbH & Co., Karlsruhe, Germany; kit 180–01 to –10) were tested. Amplifications were carried out in 25 µl volumes containing 10 mM Tris–HCl, pH 8.8 at 25°C, 1.5 mM MgCl2, 150 mM KCl, 0.1% Triton X–100 (1 x PCR–buffer, Finnzymes, Espoo, Finland), 0.5 U DNAzyme™ II Polymerase (Finnzymes, Espoo, Finland), 1 mM of primer, 0.2 mM dNTPs (Amresco, Solon, USA) and 100 ng template DNA. The DNA was amplified in a thermal cycler (Progene 02, Techne, Cambridge, UK) programmed for an initial denaturation of 120 s at 94°C, followed by 45 cycles of 30 s at 94°C, 60 s at 42°C, and 120 s at 72°C. The final primer extension step was extended to 10 min at 72°C. PCR products were analysed by electrophoresis on 1.4% agarose gel.

Statistical analysis

DNA fragments generated by RAPD analysis were scored as an individual locus and each locus represents a two–allele system. The banding patterns were converted into a binary matrix based on presence (1) or absence (0) of RAPD markers. Bands with the same size were regarded as homologous (Grosberg et al., 1996) and differences in intensity were not considered. Faint or irregularly appearing bands were not included (Williams et al., 1993). The estimation of allele frequency for dominant markers presents some statistical difficulties. However, estimates of allele frequency can generally be applied in highly polymorphic dominant markers (Krauss, 2000; Tero et al., 2003). Similarity indices (S) according to Nei & Li (1979) were calculated, where \( S = 2m_{xy} / (m_x + m_y) \), with \( m_{xy} \) being the number of shared markers between two individuals, while \( m_x \) and \( m_y \) are the number of markers for each individual. The mean values of the similarity index and the mean genetic distances (Nei, 1972) were statistically compared using Mann–Whitney \( U \)–test (SPSS 10.0.7 for Windows, SPSS Inc., Chicago, USA).

Because the reintroduced beaver populations were not expected to have Hardy–Weinberg–Equilibrium, the method by Lynch & Milligan (1994) was not appropriate in this case. Therefore, we alternatively treated the multilocus phenotype as a haplotype in the programs POPGENE 1.31 (Yeh & Boyle, 1997) and ARLEQUIN 2.000 (Schneider et al., 2000), as suggested by Huff et al. (1993), Holsinger et al. (2002) and Jimenez et al. (2002). Intrapopulational genetic diversity \( h \) (Nei,

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5´–3´)</th>
<th>Size (bp)</th>
<th>Castor fiber</th>
<th>Castor canadensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roth 180–01</td>
<td>GCACCCGACG</td>
<td>250–1,600</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Roth 180–04</td>
<td>CGCCCGATCC</td>
<td>300–1,900</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Roth 180–06</td>
<td>GCACCGAGGG</td>
<td>400–1,080</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Roth 180–08</td>
<td>CGCCCTCAGC</td>
<td>400–1,100</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>55</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 2. RAPD primers used in the study, size and number of amplified DNA–fragments for each primer, number of polymorphic bands, and percentage of polymorphic bands in brackets: B. Bands in total; P. Polymorphic bands (%).
Results

The four best performing primers (Roth 180–01, –04, –06 and –08), according to reproducibility and polymorphic banding pattern, were chosen for all samples. Sequences and detailed information on the RAPD primers used are listed in table 2. PCR–products ranged from 250 bp to 1900 bp. In total, 55 reproducible bands were obtained for C. fiber, 36 of which (65.5%) were polymorphic. C. canadensis showed a total of 43 reproducible bands, fifteen of these markers (34.9%) were polymorphic. We found one diagnostic RAPD marker (180–04 1000 bp) that was monomorphic for C. fiber and absent in the C. canadensis samples. Furthermore, we identified four other diagnostic markers (180–01 250 bp, 180–04 1350 bp, 180–08 450 bp, 180–08 400 bp) that were only fixed in the C. fiber samples.

Similarity indices (S) within and among the C. fiber populations were generally high (table 3). Overall, the similarity indices within the beaver populations (mean: 0.891 ± 0.032 SD) were not significantly higher (U–test: P = 0.13) than the genetic similarity among the C. fiber populations (mean: 0.850 ± 0.012 SD), while the similarity indices were significantly higher among these populations and the analysed C. canadensis samples (mean: 0.750 ± 0.009 SD; U–test: P = 0.05). Within the populations, individuals from Bavaria showed the highest similarity, whereas the Austrian population showed the lowest value for genetic similarity. The comparison between the Austrian beavers and samples from the Elbe River showed the lowest similarity among the C. fiber populations.

The three C. fiber populations showed a moderate genetic differentiation (AMOVA: 18.2% of the variation among the three analysed populations, table 4). Furthermore, adding C. canadensis as a second group, the analysis of the genetic structure of the populations by AMOVA analysis revealed that over 44% of the genetic variance was situated within the single populations. However, there was still a significant differentiation between population units (> 9%), suggesting slightly phenotypic variation among the C. fiber populations. The portion of molecular variances among the C. fiber populations and the C. canadensis individuals amounted to over 45%. All three levels are highly significant (P < 0.001).

The average intrapopulational genetic diversity h calculated following Nei (1987) within C. fiber populations was 0.183, whereas the samples from Bavaria showed a lower value, and the individuals from Austria the highest value (table 5). The C. fiber populations showed overall low genetic distances (table 5) and again C. canadensis was significantly distinguished from the C. fiber populations (mean: 0.291 ± 0.046 SD; U–test: P = 0.046).

The consensus tree (fig. 2) that resulted from the Neighbor–Joining cluster analyses of all beaver samples revealed three groups: the C. canadensis samples, three C. fiber samples (A2, A3 and A7)
Table 4. Results of the hierarchical analysis of molecular variance (AMOVA) within and among the beaver populations sampled, using 55 RAPD markers. Notice that the analysed C. canadensis individuals were regarded as one population. Among populations within groups are the individuals of C. canadensis as one group and the three C. fiber populations as the second group. The level of significance is based on 1,023 random permutations: Df. Degree of freedom; Sq. Sum of squares; Vc. Variance components; Pv. Percentage of variation; Fi. Fixation indices (NC. Not computed; \( f_{CT} \). Correlation of random RAPD phenotypes within groups relative to total; \( f_{SC} \). Correlation within populations relative to group; \( f_{ST} \).Correlation within populations relative to total).

Table 4. Resultados del análisis jerárquico de la varianza molecular (AMOVA) dentro y entre las poblaciones de castor muestreadas, utilizando los marcadores RAPD 55. Obsérvese que los individuos analizados de C. canadensis se consideraron como una población. En el análisis entre poblaciones, los individuos de C. canadensis se consideraron como un grupo, y las tres poblaciones de C. fiber como un segundo grupo. El nivel de significación se basa en 1.023 permutaciones al azar: Df. Grados de libertad; Sq. Suma de cuadrados; Vc. Componentes de la varianza; Pv. Porcentaje de variación; Fi. Índices de fijación (NC. No computado; \( f_{CT} \). Correlación entre los fenotipos RAPD tomados al azar dentro de los grupos en relación con el total; \( f_{SC} \). Correlación dentro de las poblaciones en relación con el grupo; \( f_{ST} \). Correlación dentro de las poblaciones en relación con el total).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sq</th>
<th>Vc</th>
<th>Pv</th>
<th>P-value</th>
<th>( f_{CT} )</th>
<th>( f_{SC} )</th>
<th>( f_{ST} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three C. fiber populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among C. fiber populations</td>
<td>2</td>
<td>28.28</td>
<td>0.974</td>
<td>18.23</td>
<td>&lt; 0.0001</td>
<td>( f_{ST} = 0.1823 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within C. fiber populations</td>
<td>28</td>
<td>122.30</td>
<td>4.368</td>
<td>81.77</td>
<td>NC</td>
<td>NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two groups (beaver species)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>1</td>
<td>40.98</td>
<td>4.512</td>
<td>45.97</td>
<td>&lt; 0.0001</td>
<td>( f_{CT} = 0.4597 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>2</td>
<td>28.28</td>
<td>0.978</td>
<td>9.97</td>
<td>&lt; 0.0001</td>
<td>( f_{SC} = 0.1845 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within populations</td>
<td>31</td>
<td>134.05</td>
<td>4.324</td>
<td>44.06</td>
<td>&lt; 0.0001</td>
<td>( f_{ST} = 0.5594 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

from the Austrian population and the remaining C. fiber samples from Austria, Bavaria and from the Elbe River. Bootstrapping supported these groups.

To gain more detailed insight into the multidimensional relationships among individuals of C. fiber and C. canadensis, we constructed a three-dimensional plot of the PCA from the 35 RAPD phenotypes (fig. 3). The first three principal components accounted for 43.1%, 10.3% and 9.6% (= 63.0%) of the total variance. Among the other 17 principal components greater than zero, none accounted for more than 5.3% of the variation. Individuals of C. canadensis were clearly separated from the individuals of the C. fiber populations. Additionally, a small group of three Austrian beaver samples was detected in an intermediate range between the C. canadensis and the C. fiber clusters, confirming the results above. No separation between individuals from the Elbe River, Bavaria or Austria was detected among the remaining C. fiber samples.

Discussion

This paper reports initial findings on the genetic structure in populations of Castor fiber in Germany and Austria. The genetic similarity (S) of the three European populations analysed was relatively high within populations (mean values ranging from 0.853 to 0.932) and among populations (from 0.744 to 0.860). C. canadensis samples showed a similar small genetic
Fig. 2. Neighbour–joining tree based on Nei’s (1972) genetic distance among all analysed beaver samples. Bootstrap support of the nodes (1,000 permutations) is only included if they exceed 50%. The *C. canadensis* sample C1 served as outgroup.

Fig. 2. Árbol filogenético de unión de vecinos ("neighbour–joining") basado en la distancia genética de Nei (1972) entre todas las muestras de castor analizadas. Sólo se incluye el muestreo ("bootstrap") soporte de los nodos (1,000 permutaciones) si superan el 50%. La muestra C1 de *C. canadensis* sirvió de grupo de comparación ("outgroup").
variation, probably because our sample was small. Lizarralde et al. (2008) have recently analysed a total of 30 specimens (with 5 specimens from Alaska as an outgroup) of *C. canadensis*, which was introduced into Isla Grande de Tierra del Fuego, Argentina in 1946. They found a high genetic variation in partial sequences of *Cytochrome b*, 12S rRNA genes and the main non-coding D–loop region.

We did not find population specific RAPD markers for *C. fiber*, but there was a clear difference in the banding pattern (in form of "diagnostic markers") in *C. canadensis* individuals. Tamate et al. (1995) interpret the absence of population–specific markers as evidence of kinship between the individuals of the different populations. Our findings agree with previously published data on protein polymorphisms of American beavers (Hoppe et al., 1984) and with allozyme analysis of reintroduced *C. fiber* populations from Kirov and Novosibirsk in Russia (Milishnikov & Savel'ev, 2001; Milishnikov, 2004). These studies revealed only relatively low levels of enzyme variation, and genetic differences between the analysed beaver populations in all studied loci were very small. Using DNA fingerprinting population genetic studies of reintroduced Scandinavian beavers displayed a similar low heterogeneity, with a mean similarity coefficient of 0.80, and only monomorphic MHC loci (Ellegren et al., 1993). Kappe et al.’s (1997) studies on harbour seal subspecies (*Phoca vitulina* ssp.) with multilocus DNA fingerprinting by revealed similar results. The subspecies *P. vitulina vitulina* also revealed high values of similarity coefficients both within and among the analysed populations of the Dutch Wadden Sea and the east coast of Scotland (*S* = 0.79 to 0.87 and 0.74–0.79 respectively), as a result of a severe bottleneck during the last glaciation.

Our results also agree with Durka et al. (2005) and Ducroz et al. (2005) who analysed the nucleotide variation in the mitochondrial DNA control region (mtDNA CR) from 152 specimens of *Castor fiber* from 39 localities in France, Germany, Norway, Poland, Lithuania, Russia and Mongolia. Over this large geographical scale, they found an extreme genetic structuring as the result of an apparent cessation of gene flow. As
in our study, they also found no or only a little genetic variation for the analysed beaver populations from Central Europe (beaver populations from Germany, France and Norway clustering together and distinct from populations east of the Oder and Vistula Rivers). On the intrapopulational level the sequence variation of the assayed fragment of the mtDNA CR within the relict Castor fiber populations was also very low. The authors conclude that this very low level of intrapopulational variation may be attributed to a recent bottleneck.

In our study, there was no significant differentiation among the original local beaver population of the Elbe River and the reintroduced animals of the Danube River system and the overall genetic similarity was relatively high. No specific bottleneck effect was detected, and the overall within-species genetic variation was low. It stands out, however, that the Austrian population, established from individuals of different origins, showed the lowest genetic similarity among all Castor fiber populations, displaying a relatively high genetic heterogeneity of the reintroduced individuals and no evidence for a founder effect. These results are consistent with the assumption that loss of heterozygosity is very slow and depends on the population size and the length and the depth of a potential population reduction (Amos & Balmford, 2001). The overall high genetic similarity of Castor fiber might be due to other factors, such as post-glacial colonisation, and it does not seem to reduce recolonisation success.

Regarding the cluster analysis and the PCA in more detail, we propose that the three Austrian beavers, grouped among the other Castor fiber and Castor canadensis samples, show a genetic structure in between the two species. These results suggest that these three intermediate individuals are possible offspring from reintroduced eastern Castor fiber subspecies or perhaps hybrids between different beaver subspecies. This is also supported by the genetic diversity of the analysed populations, as only the beaver samples from the reintroduced population in Austria show a relatively high value, compared to the other two populations. Hybridisation between Castor fiber and Castor canadensis seemed to be unlikely because breeding experiments were not successful in captivity (Djoshkin & Safonow, 1972; Kuehn et al., 2000; Halley & Rosell, 2002) and the karyotypes of the two species are different (Lizarralde et al., 2004).

For long-term conservation strategies, the autochthonous Castor fiber populations might be the hot spots of beaver conservation, as Durka et al. (2005) suggested. New reintrogression programs of beavers in Europe should be accompanied by a clear genetic identification of reintroduced individuals so as to prevent reintroduction of Castor canadensis in Europe. Further analyses using co-dominant techniques in a larger sample of individuals should be performed to confirm these first results of an interspecific gene flow.

Acknowledgments

This project was financially supported by the “Hessische Gesellschaft für Ornithologie und Naturschutz” (HGON) in the context of the “Licher Stipendium”. We thank G. Schwab (Mariaposching, Germany), J. Sieber (Wien, Austria) and D. Heidecke (Halle, Germany) for providing samples of Castor fiber and Castor canadensis.

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