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

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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* Linnaues, 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 semejanza genética en la especie *C. fiber*, no hallándose evidencias de una estructura subespecífica en las poblaciones estudiadas.

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

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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 Europewere 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 watershedin Bavaria. In total, 120 beavers (*C. fiber*) of different origins were reintroduced along the rivers Danube and Inn between 1966 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 & Baveco, 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; Vucetich 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;

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.

Pop	Origin	Ss	Species/Subspecies
Austria	Austria/Danube	7	*Castor fiber* spp.
Canada	Austria/Danube	1	*Castor canadensis*
Elbe	Germany/Kinzig–Prims	3	*Castor fiber albicus*
Elbe	Germany/Elbe	8	*Castor fiber albicus*
Bavaria	Germany/Danube	13	*Castor fiber* spp.
Canada	Germany/Danube	3	*Castor canadensis*

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.

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

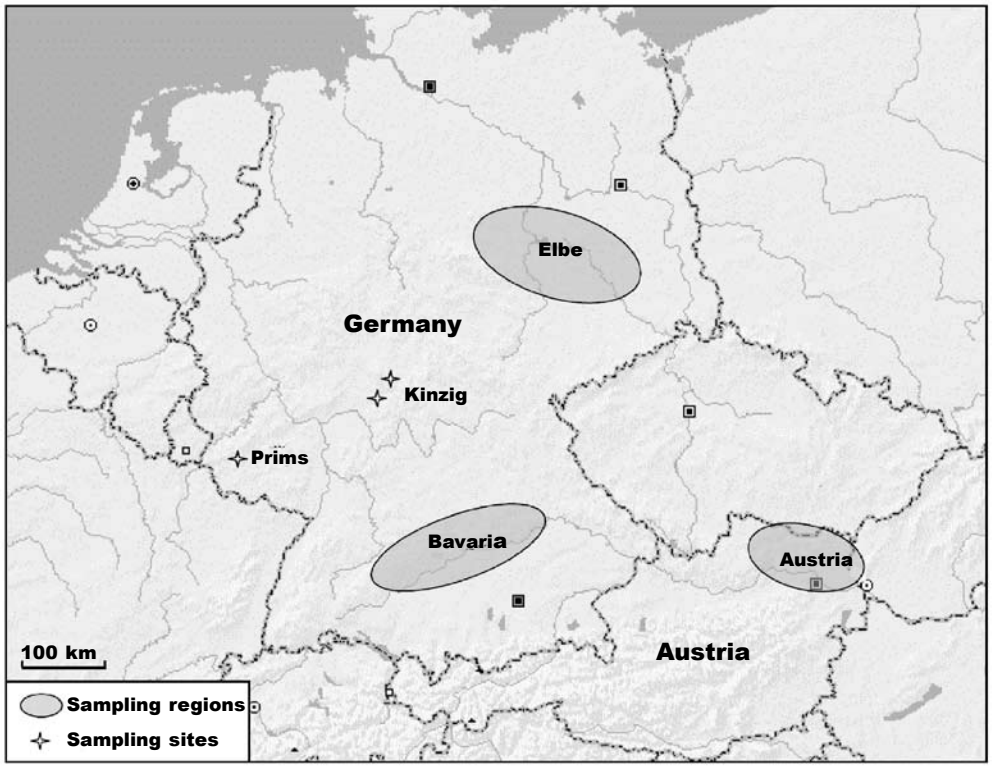

Fig. 1. Geographic distribution of the sampling sites (individual beaver samples marked with an asterisks were assigned to the Elbe population) in Austria and Germany.

Fig. 1. Distribución geográfica de los lugares de muestreo (las muestras de castores individuales marcadas con un asterisco se asignaron a la población del Elba) en Austria y Alemania.

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 (%).

Tabla 2. RAPD primers utilizados en el estudio, tamaño y número de los fragmentos de ADN amplificados para cada primer, número de bandas polimórficas, y entre paréntesis porcentaje de bandas polimórficas: B. Bandas totales; P. Bandas polimórficas (%).

			Castor fiber		*Castor canadensis*	
Primer	Sequence (5´–3´)	Size (bp)	B	P (%)	B	P (%)
Roth 180–01	GCACCCGACG	250–1,600	13	9 (69.2)	8	4 (50.0)
Roth 180–04	CGCCCGATCC	300–1,900	17	11 (64.7)	13	5 (38.5)
Roth 180–06	GCACGGAGGG	400–1,080	12	8 (66.7)	12	2 (16.7)
Roth 180–08	CGCCCTCAGC	400–1,100	13	8 (61.5)	10	4 (40.0)
Total			55	36 (65.5)	43	15 (34.9)

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 H_2O. 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 $MgCl_2$, 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 gels in 1 x TBE buffer (89 mM Tris–borate, 2 mM EDTA, pH 8.0) for approximately 4 hours at 70 V (55 mA), visualized by staining with ethidium bromide and photographed under UV radiation with a Polaroid type 667 film (Polaroid, Waltham, USA). PCR–conditions were optimized following Bielawski et al. (1995). In order to ensure reproducible results and minimise errors one beaver sample was randomly chosen and amplified with each PCR as positive control, another sample was amplified twice in the same PCR. Duplicate amplifications (PCR runs) were conducted for each sample. Bands which could not be reproduced in both assays were not considered for analysis.

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 3. Similarity index *S* (Nei & Li, 1979) within populations (in *italics*) and between populations. Means are given with their standard deviations.

Tabla 3. Índice S de similitud (Nei & Li, 1979) dentro de las poblaciones (en cursiva) y entre las poblaciones. Se dan las medias junto con sus desviaciones estándar.

	Austria (n = 7)	Elbe (n = 11)	Bavaria (n = 13)	*C. canadensis* (n = 4)
Austria	*0.853 ± 0.053*			
Elbe	0.837 ± 0.055	*0.889 ± 0.051*		
Bavaria	0.852 ± 0.050	0.860 ± 0.069	*0.932 ± 0.029*	
C. canadensis	0.746 ± 0.078	0.744 ± 0.033	0.761 ± 0.022	*0.891 ± 0.059*

1987) and genetic distances *D* (Nei, 1972) among the analysed populations were evaluated using POPGENE. Population structure was checked by an analysis of molecular variance (AMOVA; Excoffier et al., 1992) using ARLEQUIN. Standard variance components and *f*–statistics were calculated using AMOVA. We performed this analysis first with the *C. fiber* samples only, and in the next step we included the *C. canadensis* as a separated outgroup to the AMOVA analysis.

We produced a set of 1000 genetic distance (Nei, 1972) matrices by bootstrapping (with randomised data entry) over all RAPD loci using the program RAPDDIST 1.0 (Black, 1996). Phylogenetic relationships (majority rule consensus tree) between all individuals were constructed based on a Neighbor–Joining algorithm (Saitou & Nei, 1987) using the NEIGHBOR and CONSENSE program in PHYLIP 3.57c (Felsenstein, 1995). In addition, the 0/1–matrix was subjected to a principal component analysis (PCA; STATISTICA 5.0, StatSoft, Tulsa, USA). The PCA achieves an ordination of the individuals according to the presence or absence of RAPD markers along principal component axes (Manly, 1994).

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. fibe*r 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).

Tabla 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).*

Source of variation	Df	Sq	Vc	Pv	*P*–value	Fi
Three C. fiber populations						
Among *C. fiber* populations	2	28.28	0.974	18.23	< 0.0001	f_{ST} = 0.1823
Within *C. fiber* populations	28	122.30	4.368	81.77	NC	NC
Two groups (beaver species)						
Among groups	1	40.98	4.512	45.97	< 0.0001	f_{CT} = 0.4597
Among populations within groups	2	28.28	0.978	9.97	< 0.0001	f_{SC} = 0.1845
Within populations	31	134.05	4.324	44.06	< 0.0001	f_{ST} = 0.5594

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

Table 5. Genetic distance *D* (Nei, 1972) among the analysed populations of *C. fiber* and *C. canadensis* (below diagonal, *italic*) and intrapopulational genetic diversity *h* (Nei, 1987): A. Austria; E. Elbe; B. Bavaria.

Tabla 5. Distancia genética D (Nei, 1972) entre las poblaciones analizadas de C. fiber *y* C. canadensis *(por debajo de la diagonal, en cursiva) y diversidad genética intrapoblacional h (Nei, 1987): A. Austria; E. Elba; B. Baviera.*

Pop	A	E	B	*h*
Austria				*0.225*
Elbe	*0.050*			0.177
Bavaria	*0.056*	*0.071*		0.111
C. canadensis	*0.238*	*0.318*	*0.318*	0.142

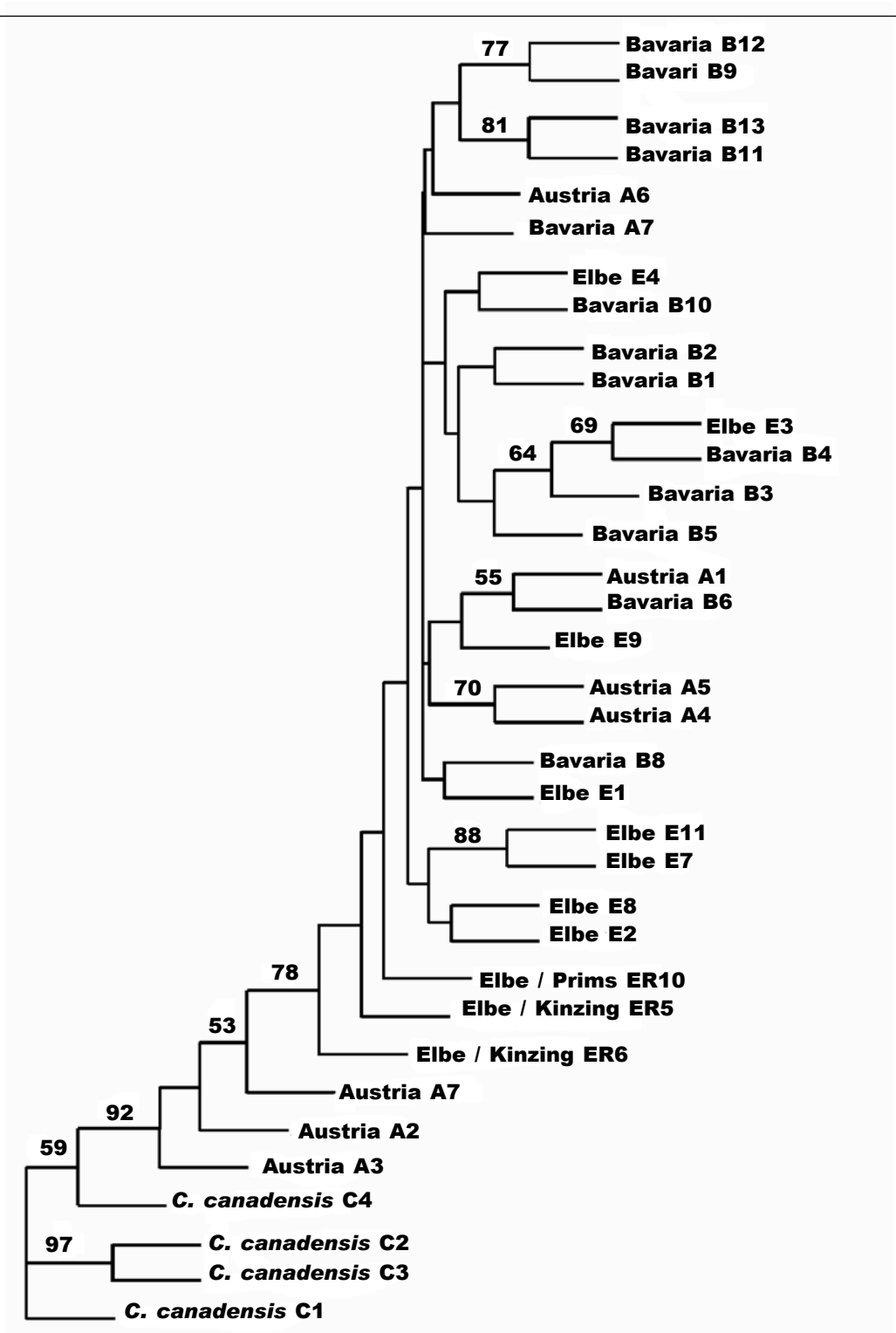

Fig. 2. Neighbour–joining tree based on Nei's (1972) genetic distance among all analysed beaver samples. Bootstrap support of the nodes (1,000 permutations) is only included if they exceed 50%. The *C. canadensis* sample C1 served as outgroup.

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").*

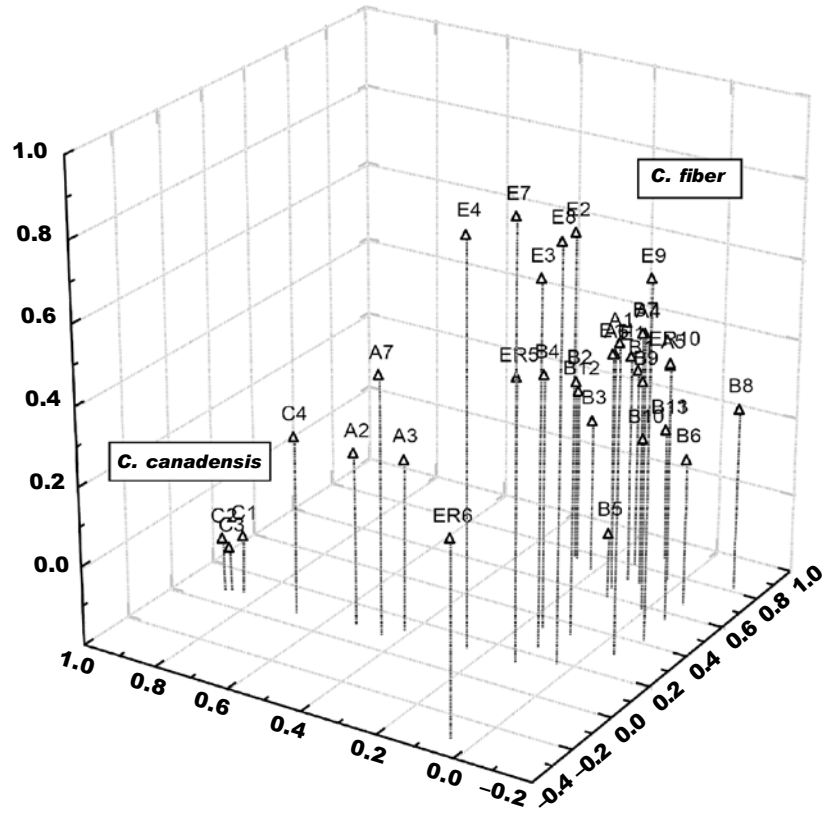

Fig. 3. Principal components analysis (Varimax normalised factor rotation) of the binary RAPD data of the different beaver samples: C. *C. canadensis*; E. *C. fiber* from Elbe; ER. Kinzig and Prims (reintroduced samples from Elbe); A. Austria; B. Bavaria.

Fig. 3. Análisis de componentes principales (rotación de factores normalizados Varimax) de los datos RAPD binarios de las distintas muestras de castores: C. C. canadensis*; E.* C. fiber *del Elba; ER. Kinzig y Prims (muestras del Elba reintroducidas); A. Austria; B. Baviera.*

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 *C. 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 *C. 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 *C. fiber* and *C. 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 *C. 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 *C. fiber* and *C. 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 *C. fiber* populations might be the hot spots of beaver conservation, as Durka et al. (2005) suggested. New reintroduction programs of beavers in Europe should be accompanied by a clear genetic identification of reintroduced individuals so as to prevent reintroduction of *C. 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.

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