Journal of Mammalogy, 86(6):1059–1067, 2005

GENETIC VARIATION AND POPULATION STRUCTURE OF THE EURASIAN BEAVER *CASTOR FIBER* IN EASTERN EUROPE AND ASIA

JEAN-FRANCOIS DUCROZ, MICHAEL STUBBE, ALEXANDER P. SAVELJEV, DIETRICH HEIDECKE, RIVČAN SAMJAA, ALIUS ULEVIČIUS, ANNEGRET STUBBE, AND WALTER DURKA*

UFZ-Centre for Environmental Research Leipzig–Halle, Department of Community Ecology, Theodor-Lieser-Strasse 4, 06120 Halle (Saale), Germany (JFD, WD)
Institute of Zoology, Martin-Luther-University Halle–Wittenberg, Domplatz 4, 06108 Halle (Saale), Germany (MS, DH, AS)
Prof. B. M. Žitkov Russian Research Institute of Game Management and Fur Farming of the RAS, Engels 79, 61000 Kirov, Russia (APS)
National University of Mongolia, Ulaan-Baatar, Mongolia (RS)
Institute of Ecology, Vilnius University, Akademijos 2, 2600 Vilnius, Lithuania (AU)

The Eurasian beaver, *Castor fiber* L., suffered extreme demographic reduction through overhunting until the end of the 19th century. However, active protection measures have led to a powerful recovery in range and population numbers. The vast majority of beavers (83%) now occur in the former Soviet Union. The present study investigates the geographic distribution of genetic variation of *C. fiber* in this eastern part of the species range (former Soviet Union and Mongolia), with special emphasis on small isolated populations of the Asian subspecies *C. fiber pohlei*, *C. fiber tuvinicus*, and *C. fiber birulai*. The analysis yielded 12 different haplotypes, all of which were population specific. Results indicate that *C. fiber* displays great population structuration ($F_{ST} = 0.985$), coupled with an overall low level of genetic divergence (mean number of pairwise differences 7.262 ± 3.435). In particular, the autochthonous populations in Mongolia or Siberia do not appear significantly different from samples from the European part of Russia, despite the great geographical distance. *C. f. birulai* appears as the most divergent member, a fact that could result from its longer genetic isolation in an enclosed watershed. Examination of our data suggests a single recent origin of the present beaver population in eastern Europe and Asia.

Key words: *Castor fiber*, conservation, genetic diversity, mitochondrial DNA, phylogeography

The Eurasian beaver, *Castor fiber* L., 1758, was formerly widely distributed across the deciduous and coniferous forested regions of Eurasia, as well as suitable riparian habitats in the tundra and steppe zones. Its historical range extended from the Iberian Peninsula and Great Britain to eastern Siberia (Djoshkin and Safonov 1972). The species was a key species of the Eurasian temperate forest fauna, notably because of its major environmental impact through the flooding of low-lying areas and felling of mature trees (Danilov and Kan'shiev 1983; Zahner 1997).

However, overhunting, primarily directed to collection of its highly prized fur and castoreum glands, together with reduction of its habitat, caused a dramatic decline in Eurasian beaver populations (Nolet and Rosell 1998). Such was the extent of the extirpation that by the middle of the 19th century the species was virtually on the verge of extinction (Heidecke 1986; Lavrov 1983). It is estimated that at that time, a total of only approximately 1,200 individuals may have survived (Nolet and Rosell 1998) in remote regions where they could escape hunting pressure, most of them in the former Russian Empire. In this eastern part of its distribution range, the species managed to survive in 5 separate regions (Lavrov 1983; Zharkov and Sokolov 1967; Fig. 1).

One group survived in Belarus and northern Ukraine, along 3 tributaries of the Dnepr (Sož, Bjarėzina, and Prypjac'), and possibly in the upper course of the Nëman water system (Lavrov 1983; Fig. 1). This group is usually considered to represent a distinct subspecies, labeled *C. f. belorussicus*, *C. f. belarusicus*, or *C. f. vistulanus* (Heidecke 1986; Pucek 1981). *Castor vistulanus* Matschie, 1907, is the oldest name available, but beavers went extinct in the region of origin of the specimen used for its description. As pointed out by Gabryś and Ważna

* *Correspondent: walter.durka@ufz.de*

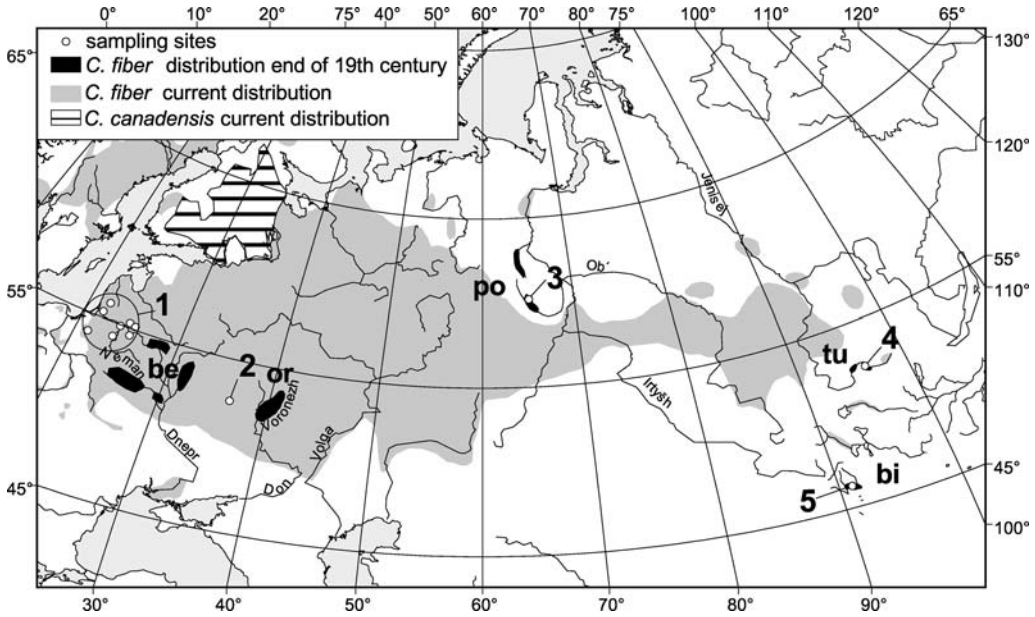

FIG. 1.—Location of the sampling sites (numbered 1–5) for the specimens of *Castor fiber* analyzed in this study. Current range of *C. fiber* and *C. canadensis* depicted according to Saveljev (2003) with additions from Halley and Rosell (2002). Aboriginal populations remaining at the end of the 19th century are labeled: *C. f. belorussicus* (be), *C. f. orientoeuropaeus* (or), *C. f. pohlei* (po), *C. f. tuvinicus* (tu), and *C. f. birulai* (bi).

(2003), *belarusicus* is a nomina nuda, and so *C. f. belorussicus* will thus be used in the present paper to refer to this form. Similarly, the population that avoided extirpation along the Voronež River, in the northern Don Basin (Fig. 1) will be referred to as *C. f. orientoeuropaeus* (Lavrov 1981).

The 3 remaining regions where beavers survived the 19th century population bottleneck are situated east of the Ural Mountain Range (Fig. 1). Some animals managed to escape extermination in western Siberia, in the basins of the Sos'va and Konda rivers, 2 tributaries of the Ob' (Saveljev 2002). They constitute the subspecies *C. f. pohlei* (Serebrennikov 1929). Another small population survived in the upper course of the Jenisej River, in the Republic of Tyva (south-central Siberia), and was considered by Lavrov (1969) to warrant subspecific status (*C. f. tuvinicus*). The 5th subspecies, *C. f. birulai*, survived in southwestern Mongolia and neighboring China (Xinjiang), along the Bulgan-gol in the Dzungarian Gobi (Serebrennikov 1929; Stubbe and Dawaa 1986), in a water basin without outlet to the sea.

Within all these populations or subspecies, numbers of individuals at the end of the 19th century were extremely low, about 290 for *C. f. belorussicus* (Lavrov and Lavrov 1986), 70 for *C. f. orientoeuropaeus* (Stubbe and Romashov 1992), 300 for *C. f. pohlei*, 30–40 for *C. f. tuvinicus* (Lavrov and Lavrov 1986), and 300 for *C. f. birulai* (Lavrov and Lu 1961; Stubbe et al. 1991). Fortunately, preservation measures and the establishment of a ban on hunting enabled the survival of these relict populations and the later spreading of *C. fiber* via both natural recolonization and man-induced translocations (Nolet 1996). In the last decades, this phenomenon has even experienced an exponential increase, and population size is currently estimated at a minimum of 593,000 (Halley and Rosell 2002), the overwhelming majority of which occur in the former Soviet Union (492,000). Expansion is continuing at a steady pace, and it can be expected that Eurasian beavers will soon reoccupy most of their historic range.

Because of this specific population history characterized by a recent and very strong population bottleneck, it is probable that *C. fiber* has suffered from a drastic loss in genetic diversity, a factor that could potentially affect the viability of present populations and lead through inbreeding and genetic drift to severe conservation problems (Avise 1994). A substantial part of the initial gene pool of the species has surely been lost forever. However, as judging from the present expansion of *C. fiber* in many areas (e.g., Fustec et al. 2001), the remaining part was significant enough to sustain dynamic demographic recovery (Halley and Rosell 2002). Reduced genetic diversity, coupled with a founder effect, could nevertheless be an important factor to explain reduced growth in certain populations (Nolet and Baveco 1996). In any case, the genetic aspects and consequences of this bottleneck effect have so far been little investigated with genetic studies remaining extremely rare (Ellegren et al. 1993; Kohler et al. 2000; Milishnikov et al. 1994, 1997; Milishnikov and Saveljev 2001). In particular it is still unclear how variable the different beaver populations are, with analyses yielding conflicting results as to levels of genetic differentiation. Ellegren et al. (1993) observed relatively little genetic differentiation when using data from DNA fingerprinting and the major histocompatibility complex, whereas Milishnikov and Saveljev (2001) and Milishnikov et al. (1994, 1997) reported substantial genetic variation in beaver populations from the Russian Federation and Belarus when using allozyme markers.

The aim of the present paper was thus to investigate genetic diversity and structure among populations of *C. fiber* in the eastern part of its distribution range (former Soviet Union and Mongolia), home to the biggest beaver populations, both now

and at the end of the 19th century. Our main objectives were to evaluate genetic diversity within and among populations, to assess geographical partitioning, and to test the genetic basis of the current subspecific arrangement. To address these goals, we targeted a hypervariable partial sequence of the mitochondrial DNA (mtDNA) control region (or D-loop) that had already proven suitable for investigations at the population level in various Palearctic mammal genera such as *Apodemus* (Koh et al. 2000; Nemirov et al. 2002), *Canis* (Randi et al. 2000; Valiere et al. 2003; Vilà et al. 1999), *Lutra* (Cassens et al. 2000), and *Ursus* (Marshall and Ritland 2002; Matsuhashi et al. 1999; Wooding and Ward 1997).

The process of creating artificial populations by hybridizing animals from different origins has been so extensive in most of the current range of *C. fiber* in Russia and neighboring countries (Djoshkin and Safonov 1972; Saveljev 2003) that it is probably already impossible to address the issue of intraspecific taxonomy in the case of *C. f. belorussicus* and *C. f. orientoeuropaeus*. In this context, maintaining the subspecific distinction appears somehow illusory, and it is more appropriate to simply refer to these animals as *C. f.* ssp.

However, in the case of the 3 Asian subspecies, *C. f. pohlei*, *C. f. tuvinicus*, and *C. f. birulai*, populations have so far remained isolated from the stock of mixed geographical forms composing the bulk of beaver communities in eastern Europe and Asia, so that it can be assumed that their current genetic diversity mirrors the initial genetic pool. Contrary to what happened to *C. f. belorussicus* and *C. f. orientoeuropaeus*, these indigenous populations have not experienced any significant growth in numbers since the end of the 19th century, and more probably a decline in the case of *C. f. pohlei* and *C. f. tuvinicus* (Lavrov and Lavrov 1986; Saveljev et al. 2000). Information on the genetic content of these demographically relatively stable populations could thus give us insights into the genetic structure of *C. fiber* before its later expansion and reintroduction. Such data could then be used for comparison with introduced or mixed beaver populations. Of course, data on genetic variation in these relict Asian forms also could help us to address the question of the validity of their current taxonomic status.

Finally, genetic information would be of the highest interest to monitor present populations as well as further reintroduction programs, both to protect the integrity of genetically differing units and to ensure successful reintroductions by avoiding the negative impact of inbreeding.

Materials and Methods

Specimens examined.—Our analysis includes a total of 81 specimens of *C. fiber* from 12 locations in Lithuania, Mongolia, and Russia (Table 1; Fig. 1). Samples consisted of beaver tail skin or hair that was obtained from live animals captured with nets, live traps, and at night with a search light and netting from a boat. Animals were released afterward. Sampling procedures were consistent with guidelines of the American Society of Mammalogists for the capture and handling of mammals (Animal Care and Use Committee 1998). In the case of *C. f. belorussicus* and *C. f. orientoeuropaeus*, despite the proximity of some of the sampling localities to regions where these relict populations maintained themselves (southern Lithuania and the Central European part of Russia, respectively), we prefer to consider these specimens of indeterminate subspecific affiliation (''*C. f.* ssp''), because of the hybridizations that have taken place between the 2 forms.

Table 1.—Sampling sites (see Fig. 1), taxonomic status (according to Gabryś and Ważna [2003], Heidecke [1986], and Lavrov [1981]), and locality of origin with number of individuals (*n*) of *Castor fiber* investigated. Unclear taxonomic status of subspecies is indicated as ''ssp.'' (see text).

Sampling site	*n*	Subspecies	Localities
1	13	ssp. (western)	Šilute, Giedraiciai, Vilaraistis, Ukmerge, Raseinai, Žemaitija National Park, Kirsna, Kaunas (Lithuania)
2	7	ssp. (eastern)	Orël region (Russia)
3	10	*pohlei*	Konda River, Kondiskyj Zakaznik (Russia)
4	39	*tuvinicus*	Azas River, Tyva Republic (Russia)
5	12	*birulai*	Bulgan-gol, Chovd Ajmak (Mongolia)

Specimens were thus arranged into 4 subspecific groupings taken from 5 distinct populations: 13 specimens of *C. f.* ssp. from Lithuania, 7 specimens of *C. f.* ssp. from Russia, 10 specimens of *C. f. pohlei*, 39 specimens of *C. f. tuvinicus*, and 12 specimens of *C. f. birulai* (Fig. 1; Table 1). Note that in the case of the last 3 forms, these samples represent a significant portion of the actual population. The most recent number of *C. f. pohlei* is estimated at 350 (Saveljev 2002), and the current population sizes of *C. f. tuvinicus* and *C. f. birulai* can be evaluated at 100–120 and 300 (Mongolian part) individuals, respectively (Samjaa et al. 1999; Saveljev et al. 2000). In addition, 3 specimens of the related species *C. canadensis* were included in the analysis to be used as outgroup for tree reconstruction and comparison.

DNA extraction.—Total genomic DNA was extracted from small pieces of tissue or hairs preserved in 70% ethanol, and extracted by following a modification of the cetyltrimethylammonium bromide (CTAB) method described in Winnepenninckx et al. (1993). Hairs were 1st digested in a lysis buffer including 10 µl of proteinase K (0.2 mg/ml) for at least 1 h. The material was then ground in liquid nitrogen without buffer, suspended in a volume of 600 µl of 10% CTAB buffer (Winnepenninckx et al. 1993) supplemented with 0.2 mg of proteinase K, and incubated at 60°C for a minimum time of 1 h. A similar volume of 600 µl of chloroform : isoamylalcohol (24:1) was added, gently mixed, and centrifuged at 4°C for 10 min at 14,000 rpm. One microliter of RNAse (100 mg/ml) was then added to each tube and incubated for 30 min at 37°C. Four hundred microliters of isopropanol was added and left for 1–16 h at 20°C. The tubes were then centrifuged (14,000 rpm for 10 min) and the pellet was cleaned by using 70% and 100% ethanol washes and resuspended into water.

DNA amplification.—Sequences from the hypervariable domain I (Saccone et al. 1987) of the control region (D-loop) of the mitochondrial genome were isolated via the polymerase chain reaction (PCR) method, and amplification was performed by using the following pair of universal primers: Thr-L15926 (5′-CAA TTC CCC GGT CTT GTA AAC C-3′) located in the neighboring tRNA-pro gene and DL-H16340 (5′-CCT GAA GTA GGA ACC AGA TG-3′—Vilà et al. 1999).

Double-stranded PCRs were performed in 20-µl or 40-µl reaction volumes. A 20-µl PCR mix contained 0.8 U of Taq polymerase, 2 µl of 10× Taq polymerase buffer with $(NH_4)_2SO_4$, 1.5 mM of $MgCl_2$,

Table 2.—Haplotypes and haplotype frequencies within subspecies of *Castor fiber*.

Subspecies	Haplotypes		
	Code	*N*	Frequency
ssp. (western)	in2	11	0.850
ssp. (western), Kirsna, Kaunas (Lithuania)	in3	2	0.150
ssp. (eastern)	in1	7	1.000
pohlei	po1	9	0.900
	po2	1	0.100
tuvinicus	tu1	31	0.800
	tu2	3	0.075
	tu3	1	0.025
	tu4	4	0.100
birulai	bi1	2	0.167
	bi2	6	0.500
	bi3	4	0.330

200 μM of deoxynucleoside triphosphates, and 5 pmol of each primer. All PCRs used the following thermal cycling parameters: 4 min at 96°C, 35 cycles (40 s at 96°C, 45 s at 55 or 56°C, and 1 min at 72°C), plus 10 min at 72°C, in a thermal cycler. PCR products were purified by using the QIAquick PCR purification kit by QIAGEN (Hilden, Germany) and resuspended in 28 μl of water.

DNA sequencing.—All specimens were directly sequenced from the purified PCR product by using the same primers as for PCR amplification. Approximately 20 ng of double-stranded PCR product was used in cycle sequencing reactions by using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (version 1.1, Applied Biosystems, Darmstadt, Germany). All sequencing reactions were performed according to the manufacturer's instructions. The following thermal cycling parameters were used: 2 min at 96°C, 25 cycles (10 s at 96°C, 5 s at 50°C, and 4 min at 60°C), plus 10 min at 72°C. Dye terminators were removed by using the DyeEx 2.0 Spin Kit by QIAGEN. Sequencing was done on an ABI Prism Automated 310 Genetic Analyzer Sequencer (Applied Biosystems). Sequences for both strands were determined.

Sequence analysis.—The sequences obtained were visually edited, managed, and aligned by using version 1.83 of the program CLUSTAL W (Thompson et al. 1994). The alignments were then optimized manually. Insertions and deletions (indels) were introduced to minimize transversions and gaps. The sequences have been deposited in GenBank under accession numbers AY623632 to AY623646.

Net sequence divergences between specimens, as well as population structure, were estimated by using the program ARLEQUIN version 2.000 (Schneider et al. 2000). Sequence variability was assessed by looking at the number of haplotypes (*N*), haplotype diversity (*h*, the probability that 2 sequences randomly drawn from 1 population are different), and nucleotide diversity (π, the probability that 2 homologous nucleotides randomly drawn from 1 population are different). A Mantel test was performed by using the ARLEQUIN software (Schneider et al. 2000) to test the significance of the correlation (*r*) between genetic and geographical distances (1,000 permutations).

Phylogenetic relationships among haplotypes were estimated by using parsimony and distance methods. All the analyses were performed by using the software PAUP* 4.0 (Swofford 2000). Distance trees were generated by using the neighbor-joining algorithm, with the HKY85+Γ model (gamma parameter 0.5). For parsimony analyses, gaps were treated as a 5th character to account for the indels present in this noncoding region. The branch and bound search option using the default settings of PAUP 4.0 was used for tree reconstruction. Three homologous sequences of *C. canadensis* were used for tree rooting. The reliability of nodes retrieved from the trees obtained from both parsimony and neighbor-joining analyses was assessed by the bootstrap resampling method based on 1,000 random replicates.

We analyzed population structure by analysis of molecular variance (AMOVA), which divides total variance into additive components, that is, variation associated with differences within and among populations, by using ARLEQUIN (Schneider et al. 2000). The statistical significance of the *F* statistics in AMOVA was assessed by using 1,000 random permutations.

Results

Sequence divergence.—Sequence analysis of the 81 specimens of *C. fiber* analyzed revealed 12 unique haplotypes for the fragment of the control region considered. The length of the amplified fragment varied from 490 to 492 base pairs (bp). No heteroplasmy was detected, either in the length or nucleotide sequence of the fragment. After alignment, the different haplotypes differed by 1–15 substitutions, of which 2 were indels, at 24 distinct positions. All of the polymorphic sites exhibited single site substitutions only, and nearly all of the substitutions were transitions. Most variable sites were located at the 3′-end of the fragment amplified, downstream of the tandem repeats region of the control region.

The 3 sequences of *C. canadensis* differed by 1–6 mutations, and were markedly shorter (473–474 bp) than their homologous counterparts in *C. fiber*. Including these haplotypes of *C. canadensis* (*ca1*, *ca2*, and *ca3*), the total length of the alignment was 497 bp. Haplotype data, including their identifying codes, taxonomic status, number of individuals, and frequencies, are indicated in Table 2. The distribution of the variable sites among haplotypes for *C. fiber* in the 497-bp alignment analysis is indicated in Fig. 2.

Each haplotype was restricted to a unique subspecies and geographical area, and was thus diagnostic at the subspecific level (Table 2). In all populations, 1 haplotype dominated, making up at least 50% of the haplotype distribution. The highest number of distinct haplotypes (4 in *C. f. tuvinicus*) was found in the population with the largest number of specimens, but the correlation between sample size and number of haplotypes was not significant.

Divergence among haplotypes was mostly restricted to a single mutation within each population, and overall sequence divergence levels were low, especially when taking into account that this region represents one of the most variable of the entire mtDNA genome. The mean number ($\pm$ *SE*) of pairwise differences within populations ranged from 0.201 $\pm$ 0.209 in *C. f. pohlei* to 0.571 $\pm$ 0.493 in *C. f.* ssp., and the mean number of pairwise differences within *C. fiber* was 7.2 $\pm$ 3.4. This contrasts sharply with the high genetic distance observed when the 2 species *C. fiber* and *C. canadensis* are compared, with a mean value of 33.8 $\pm$ 17.4. Nucleotide diversity indices (π) were correspondingly low, varying from 0.0004 $\pm$ 0.0006 within *C. f. pohlei* to 0.0011 $\pm$ 0.0011 within *C. f.* ssp., with the mean value within the total *C. fiber* sample being 0.0148 $\pm$ 0.0078. Overall haplotype diversity (*h*) within

haplotype	nucleotide position
	1111222222333333444444
	0346145789012467112446 9
	7382040408549396589769 90
bi1	GAACTCGGCCTGGTATCATCGAAG
bi2	.....-...
bi3	-.....-...
po1	..G...AAT.C.ACG...CT-...
po2	.CG...AAT.C.ACG...CT-...
tu1	.....TAATT.A..G.T.CT-G.C
tu2	.....TAATT.A..G.T.CT-G.C
tu3	.....TAATT.A.CG.TGCT-G.C
tu4	.....TAATT.A..GGT.CT-G.C
in1	-..TGTAAT.....G...CT-.G.
in2	-..TGTAA.....G...CT-.G.
in3	-..TGTAAT...A.G...CT-.G.

Fig. 2.—Condensed dot matrix displaying haplotypes and variable sites of the 497-base pair aligned fragment of the mtDNA control region for *Castor fiber*. Haplotype codes are given on the left, and nucleotide positions are displayed at the top, with position 1 corresponding to the 1st position of the portion of the control region sequenced in this analysis; dash indicates an insertion–deletion (indel).

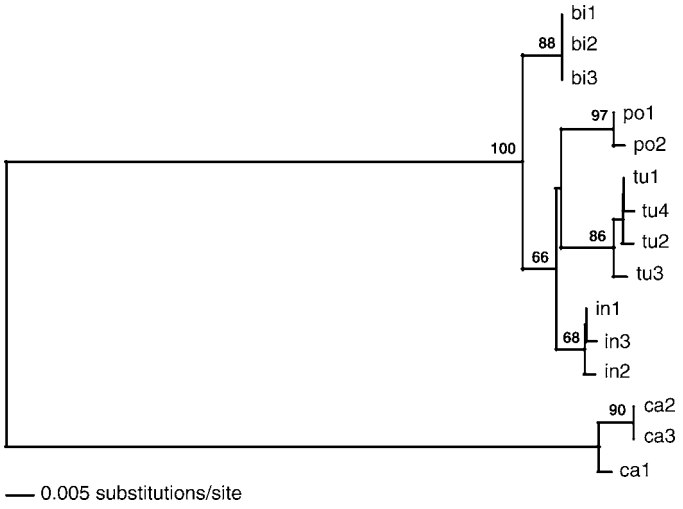

Fig. 3.—Neighbor-joining tree obtained for *Castor fiber* from HKY85 distances calculated for the 497-base pair sequence of the mtDNA control region. Bootstrap values (1,000 replicates) are given at each node.

populations ranged from 1.00 (all haplotypes identical in the Orël population of *C. f.* ssp.) to 0.33 ± 0.015 (*C. f. birulai*).

Population relationships.—The neighbor-joining tree obtained from the analysis is presented in Fig. 3. Parsimony searches yielded congruent patterns, with the provision that some relationships were left unresolved, as would be expected from the relatively small number of informative sites.

All subspecies appeared as monophyletic units (bootstrap support ranging from 75% to 99%), including specimens of *C. f.* ssp. from Lithuania and Russia, which were tightly clustered together. It appears from all analyses that *C. f. birulai* is the most basal member, mirroring its higher level of pairwise nucleotide differentiation when compared to other taxa. However, statistical support for this position of *birulai* as sister-group to the other Eurasian beavers from eastern Europe and Asia was rather low (66%), and the corresponding node was rather short (3 steps in parsimony analysis). The 3 other lineages (ssp., *pohlei*, and *tuvinicus*) are almost equally distant to each other, and their relationships are unresolved in parsimony analysis.

Population structure.—As already mentioned, all haplotypes were restricted to a single subspecies and population as defined in our analysis (Table 2). This highly heterogeneous haplotype distribution is reflected in the extremely high values obtained for genetic structuration. An AMOVA on the taxonomic and geographically based populations of *C. fiber* revealed that 96.7% of the nucleotide variation is to be found among populations.

When a Mantel test is applied to our complete data set to check for a possible correlation between genetic and geographical distances, it appears that there is no significant connection between the 2 variables ($r = 0.434$; $P > 0.152$). Interestingly, the 2 genetically most distant populations, *C. f. birulai* and *C. f. tuvinicus*, are also geographically the most closely related. The lack of correlation between geographic and genetic distances remains whatever the subsets of population compared.

Discussion

The populations of *C. fiber* examined in this study display low levels of genetic divergence, both at the intra- and interpopulation level. These low levels of genetic variability are rather close to values obtained on a slightly shorter portion of the same domain of the control region for the brown bear (Taberlet and Bouvet 1994), a species that also has undergone a severe population bottleneck at the end of the 19th century. They are markedly lower than values reported for other Palearctic taxa such as bats (Ruedi and Castella 2003), smaller rodents (Jaarola and Searle 2002), or hares (Riga et al. 1999).

It might be argued that we missed parts of the actual genetic diversity because of the relatively low number of specimens examined per population. However, in the case of the 39 specimens of *C. f. tuvinicus*, we may have surveyed as much as one-third of the extant population. At the interpopulational level, the differentiation among populations is somehow inferior to what could a priori be expected in forms considered as distinct subspecies, and distributed over such a large geographical range.

The great reduction in beaver numbers after overhunting in the 18th and 19th centuries may have contributed to the low level of genetic variability within modern populations. The population size of the aboriginal population during the bottleneck was lower than 300 animals and may have been as low as 30 individuals in *C. f. tuvinicus* (Nolet and Rosell 1998). Thus, genetic drift would have led to drastic loss of genetic variation. Low levels of variation in mtDNA frequently have been found in populations that underwent strong bottlenecks (e.g., Hellborg et al. 2002; Russello et al. 2004). However, the real extent of this anthropogenic loss of genetic variation is

difficult to assess and would require the examination of archaeological material from before the extirpation. Such a study on the similarly widely extirpated sea otters by using D-loop sequences and microsatellite markers has demonstrated that genetic variation was significantly higher before the bottleneck (Larson et al. 2002).

The high levels of genetic structure observed in our data among present populations of *C. f.* ssp., *pohlei*, *tuvinicus*, and *birulai* also is likely to result to some extent from the consequences of the population bottleneck and the survival of the species in geographically widely separated regions. In the course of the contraction and fragmentation of the range of the species the 18th and 19th centuries, intermediate populations have been lost and gene flow has ceased between the surviving populations, making the modern remaining populations appear genetically more distant than they actually were before the population decline. Similar artifacts have been evidenced in the case of fragmented populations of the South African buffalo (*Syncerus caffer caffer*—O'Ryan et al. 1998) or Australian marsupials (Houlden et al. 1996, 1999; Taylor et al. 1994).

The overall pattern of low level of divergence seems to contradict earlier investigations using allozymes that showed high genetic differentiation among Bjarėzina (Belarus), Čepca, and Voronež (European part of Russia) beavers (Milishnikov et al. 1997). The fact that allozyme markers correspond to nuclear genomic variability and that our marker is a cytoplasmatic matrilineally inherited DNA molecule might be an explanation for this discrepancy. Such a lack of correlation between mitochondrial and nuclear DNA genetic data has indeed already been observed in other studies (Avise 1994; Waits et al. 2000), and is often attributed to differences in the levels of gene flow in males and females (Avise 1994; Moritz 1994). However, there is currently no evidence for preferential dispersal of male beavers (Hartman 1997; Sun et al. 2000). Another much more likely explanation for the lack of correlation could also be genetic drift effects during very recent bottleneck and colonization events that led to pronounced local differentiation of allozyme markers (e.g., Milishnikov 2004). It would in any case be interesting to study nuclear markers (e.g., *SRY* gene and microsatellites) on the same large scale as in this study to see if the genetic pattern is confirmed.

However, our data are fully congruent with those in the study by Ellegren et al. (1993) on the major histocompatibility complex, which showed very little variability. When using allozyme markers, great similarity was reported by Milishnikov and Saveljev (2001) between 1 studied specimen of *C. f. tuvinicus* and samples from *C. f.* ssp. Genetic hybridization of *C. f. tuvinicus* with *C. f. orientoeuropaeus* was discussed as a potential cause. According to our new data, it appears that no introduction and hybridization event is necessary to explain this great similarity, which may reflect the high genetic homogeneity of *C. fiber* in eastern Europe and Asia. Examination of our data gives no indication of any hybridization of *C. f. tuvinicus* with *C. f.* ssp.

The genetically most divergent population examined in our study is *C. f. birulai*, a fact it is tempting to link to the greater historical isolation of this population. The water system now occupied by Mongolian and Chinese beavers is without outlet to the Irtyš River system and these animals have probably evolved in complete isolation from other communities for many centuries, a phenomenon that could account for their present greater genetic differentiation through genetic drift alone. One can also notice that geographic isolation seems to have had a greater impact on modern genetic patterns than water basins. Hence *C. f. birulai* and *C. f. pohlei* belong to the same drainage system (Ob'-Irtyš), but the latter species appears more closely related to the geographically closer *C. f. tuvinicus* from the Jenisej River basin.

Climatic fluctuations probably had a great influence on the genetic variability and structure of the species. The present range of the Eurasian beaver is almost entirely situated in regions that were totally unsuitable to its survival during the last glacial ages. Like many other dwellers from the modern temperate forest zones of the Palearctic, *C. fiber* experienced great fluctuations in its range and population size in the Pleistocene. A probable pattern is that successive pulses of extinctions of the northern populations during ice ages were followed by subsequent northward expansions from southern refugia during interglacials (Taberlet et al. 1998). Such taxa are usually characterized by relatively low levels of genetic differentiation at the intraspecific level, whereas populations living in refuge regions less affected by climatic changes display higher genetic differentiation.

From the evolutionary point of view, examination of our data clearly points to the genetic homogeneity of beavers from eastern Europe and Asia. If populations had been separated over a long period of time by historical barriers preventing gene flow, a pattern with geographical segregation or hierarchical nesting of the lineages along recognized boundaries, coupled with some form of morphological differentiation, might be expected (Avise 1994). However, in our case no discontinuity can be detected in the genetic distribution of the haplotypes, with the level of genetic differentiation being roughly equal among all populations investigated. This lack of clear hierarchical pattern could indicate that the modern beaver population in eastern Europe and Asia probably originated from a common postglacial colonization event, challenging the hypothesis that beavers may have reoccupied the eastern part of their range from several separate sources. Moreover, because the population of *C. f. birulai* does not appear genetically more diversified in any significant way (the usual signature for refugia), it is likely that the southern end of the Ob'-Irtyš basin, in present China and Mongolia, did not act as a refugium in the last ice age, and that the presence of *C. fiber* in this area is the result of postglacial colonization. When comparing our data on pairwise nucleotide divergence with those from other documented taxa, it also appears that the levels of divergence could fit with an origin of the different present populations as recent as the latest postglacial warming.

From the taxonomic point of view, it is rather clear from our data that the morphological differentiation that can be observed between the different subspecies (Lavrov 1983) is not matched on the genetic level. In this respect, the degree of divergence detected is below values usually reported for subspecies

differentiation. The mean pairwise genetic distance between populations is 2–3%, a value corresponding to local variation among lineages of the brown bear in western and southeastern Europe (Taberlet and Bouvet 1994) or in Japan (Matsuhashi et al. 1999) on a similar portion of the mtDNA control region. However, before drawing any firm conclusions regarding the taxonomic status of the populations of *C. fiber* in eastern Europe and Asia, it would certainly be necessary to increase our sampling effort and look at additional markers, and most importantly, to look at the comprehensive species level, that is, including western Europe. As regards our sample of composite populations of *C. f.* ssp. from Lithuania and European Russia, the sample appears extremely homogenous and undifferentiated from the genetic point of view, but we cannot rule out the possibility that only 1 of the original population gene pools was sampled (whether *belorussicus* or *orientoeuropaeus*), and that we have thus missed a significant part of the genetic variation. In this respect, it also would be necessary to increase the sample of specimens examined, and if possible to target the very regions where each form survived at the end of the 19th century.

It is to be noted that populations of *C. canadensis*, the indigenous beaver from North America, now occupy major parts of Finland and some part of Russia (Karelia, Amur River system, Kamčatka) via introductions. However, the species has never been reported to hybridize with *C. fiber* in contact zones and thus to affect the genetic content of the later (Saveljev 2003).

Our mtDNA data are also of particular relevance from a conservation point of view. It is often argued that genetic data should be included in the definition of management and conservational units. Moritz (1994) suggested that priority in conservation strategies should be directed to evolutionarily significant units, that is, sets of populations distinguished by strong phylogenetic structuring of mtDNA variation. In this respect, our results are ambiguous as regards the evolutionary value to be awarded the different subspecies and populations investigated. On the one hand, our data point to the recent origin of beaver populations from eastern Europe and Asia, probably originating from a single colonization event after the last glacial period, and to their genetic relatedness. But on the other hand, and probably largely as a consequence of the recent bottleneck and fragmentation endured by the Eurasian beaver, *C. f. pohlei*, *C. f. tuvinicus*, and *C. f. birulai* now appear as discrete genetic units, characterized by their own set of diagnostic haplotypes. The question is thus raised of the pertinence and interest of maintaining this slight genetic differentiation, and other parameters, such as the possible morphological or ecological adaptations developed by these populations. This issue is actually a current one, because hybrid beavers from the main stock of *C. f.* ssp. are expanding rapidly in western and southern Siberia, and will probably come into contact quite soon with populations of *C. f. pohlei* and *C. f. tuvinicus*. It would certainly be necessary to look on a larger scale including populations from the western part of the distribution range (France, Germany, and Scandinavia) before drawing any conclusions regarding the importance from a conservation point of view of the populations we have examined in this study.

Acknowledgments

This project was financially supported by the European Union through a Marie Curie Development Host fellowship (EVK2-CT-200-56125). We thank all the people who participated or helped in the collection of material used in this study, in particular T. A. M. Vasin from Khanty-Mansijsk (Russia) who helped collecting samples from *C. f. pohlei*, and J. Herr and the Zoo of Gelsenkirchen (Germany) for providing material of *C. canadensis*. We are grateful to I. Geier, M. Hermann, and A. Thondorf for technical help and to S. Michalski and M. Solé for advice on sequencing reaction protocols and software use, respectively. Special thanks to D. J. Halley for his kind response to requests for information, and to R. Hutterer and F. Rosell for their comments on an earlier draft of this manuscript.

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Submitted 30 September 2004. Accepted 20 April 2005.

Associate Editor was Eric A. Rickart.