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Genetics

Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers

(population bottleneck/genetic diversity/conservation genetics)

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ABSTRACT **Loss of genetic variation due to population bottlenecks may be a severe threat for the survival of endangered species. Assessment and maintenance of genetic variability are thus crucial for conservation programs related to endangered populations. Scandinavian beavers went through an extensive bottleneck during the last century due to overhunting. In Sweden the species became extirpated but in Norway extinction was avoided by legal protection. Following reintroductions of small numbers of remaining Norwegian animals in 1922–1939, the Swedish population has increased tremendously, now harboring 100,000 animals. We show here that this viable population of beavers possesses extremely low levels of genetic variability at DNA fingerprinting loci and monomorphism at major histocompatibility complex (MHC) class I and class II loci. A similar pattern was also evident among Norwegian beavers but low levels of genetic variability were not a characteristic of the species since Russian conspecifics displayed substantial DNA fingerprinting polymorphism. However, the Russian animals were monomorphic at MHC loci, indicating that the European beaver is exceptional in its low level of MHC variability. The results demonstrate that a conservation program can be successful despite low levels of genetic variation in the founder population.**

A paucity of genetic variability has been observed in several natural populations thought to have passed through extensive bottlenecks (1–7). Inbreeding depression caused by such bottlenecks may be a major concern for the survival of endangered species (8–10), and management strategies thus often aim at minimizing loss of genetic diversity (11–14). Decreased genetic variability at major histocompatibility complex (MHC) loci (15) may be particularly harmful for small populations by increasing their susceptibility to epizootics (10). MHC molecules play a central role in the immune system by presenting foreign peptides to T-effector cells. Extreme susceptibility to virus infections in cheetahs (*Acinonyx jubatus*) and in cotton-top tamarins (*Saguinus oedipus*) has been suggested to be associated with low degrees of MHC polymorphism in these species (refs. 16 and 17; see also refs. 18 and 19). In contrast, extensive MHC polymorphism has been documented in a majority of species studied, including man and other primates, mouse and other rodents, and farm animals such as cattle, sheep, goat, pig, horse, and chicken (see ref. 15 for review). There is compelling evidence that MHC polymorphism is maintained by some form of balancing selection (20–23), which most likely is related to the susceptibility to pathogens.

Once spread over the entire Scandinavian peninsula, beavers (*Castor fiber*) previously dropped significantly in numbers (Fig. 1), suffering from extensive overhunting. In Norway and Sweden the species was still abundant over large areas in the countries in the 17th century (thousands of pelts were exported annually; ref. 24), but in the 18th century reduced Swedish numbers were first reported (25). After a drastic decline, the last reports of the original Swedish population date back to the 1870s. In Norway, however, extinction was avoided by legal protection in 1845. A Norwegian population size of about 100 animals was estimated in 1888, meaning that the size is likely to have been even lower at the time of protection (26).

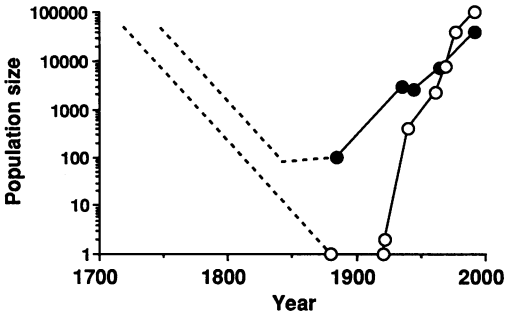

FIG. 1. Estimated size of the beaver populations in Norway (●) and Sweden (○) during the last centuries. Data are from refs. 24–27 and G.H., unpublished data. The population sizes at the time of the 18th century should be regarded as rough estimates. The small decline in the Norwegian population in 1940 was probably a consequence of illegal hunting during World War II.

About 80 Norwegian animals were released at 19 different areas in Sweden during the period from 1922 to 1939. Reintroduction was successful for, at most, 46 animals at 11 sites (2–9 animals at each site) spread over Sweden (27). After this, beavers have propagated tremendously in Sweden, the current population size being estimated at 100,000 animals (Fig. 1). The Norwegian population has shown a similar trend, although these animals began to recover earlier due to the time of protection. The rapid population growth suggests that the population collapse has not had any drastic effect on viability or fertility. Scandinavian beavers are thus an interesting model for studying the genetic consequences of a population bottleneck as well as founder effects caused by reintroduction of a small number of animals.

In this study we have investigated the genetic variability of beavers by analyzing restriction fragment patterns obtained with a set of MHC class I and class II probes and by DNA fingerprinting. DNA fingerprinting has proved to be a good

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Abbreviations: MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism; APD, average percent difference.

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indicator of the overall genome variability (28). To be able to discriminate between founder effects from the reintroductions and previous bottleneck effects, we compared the genetic variability of Swedish beavers with that of Norwegian animals. As a reference point for the amount of genetic variability in European beavers we used Russian conspecifics from the vicinity of Kirov.

MATERIALS AND METHODS

Samples. Twenty-five beavers were collected from a river system at Hällefors in southern Sweden during the period 1987–1990 (a 2000-km^2 area; 59°.40′N/13°.00′E). Six animals derived from three additional systems (two animals from each site) in Sweden (situated 30, 100, and 400 km from Hällefors, respectively) were used as controls. In 1990, 15 Norwegian beavers were collected at Kristiansand in southern Norway (an 800-km^2 area; 59°.20′N/9°.30′E), and in 1991 six animals were collected at Kirov in Russia within a 500-km^2 area (53°.30′N/36°.00′E).

DNA Analysis. DNA was prepared from frozen muscle or liver samples as described (29). Ten micrograms was digested with *Hin*fI or *Hae* III for DNA fingerprinting, and with *Taq* I or *Pvu* II for MHC restriction fragment length polymorphism (RFLP) analysis. Agarose gel electrophoresis, filter preparation, probe labeling, and hybridization conditions followed ref. 30; however, for MHC probes, 5% dextran sulfate and 0.2 mg of salmon sperm DNA per ml were included in the hybridization solution, and these filters were washed in 0.7× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate) at 58°C. For DNA fingerprinting we used Jeffreys' 33.6 and 33.15 probes (31), and the tandem repeat in the M13 wild type (32). For MHC RFLP analysis the human cDNA probes corresponding to class I (locus not determined) and class II DQA, DQB, DRB, and DPB were employed (33).

Statistics for Estimates of Genetic Variability. We used the average percent difference (APD) method to estimate the variability of DNA fingerprints and MHC RFLPs (34). APD is the mean, multiplied by 100, of pairwise comparisons where the number of differing fragments between two individuals is divided by the total number of fragments present in these individuals. For DNA fingerprints, only fragments larger than 3 kb were considered.

RESULTS

The genetic variability of Swedish beavers from Hällefors was extremely low as indicated by DNA fingerprinting with M13 and Jeffreys' 33.6 and 33.15 probes (Fig. 2*A*). APD values based on pairwise comparisons between all individuals ranged from 10.8% to 23.6% depending on probe/enzyme combination (Table 1). The individuals from Hällefors can be seen as a representative sample of Swedish animals since a similar low DNA fingerprinting variability was found in six control animals from three other Swedish sites. APD values for the control animals ranged from 12.4% to 16.7% with no obvious differentiation vis-á-vis the sample from Hällefors (inter-APDs of 13.9–22.3). Calculations of the mean band frequency in each probe/enzyme combination for the main sample revealed an average value of 0.80. This is considerably higher than band frequencies of 0.10–0.30 usually found (using M13 and Jeffreys' 33.6 and 33.15 probes) in natural populations of mammals and birds and is only matched by the low DNA fingerprint variability observed among small island populations of the California Channel Island fox (*Urocyon littoralis*) (6) and the gray wolf (*Canis lupus*) (7) and that within colonies of the eusocial naked mole-rat (*Heterocephalus glaber*) (ref. 35; in which consanguineous matings are likely to occur within colonies). Norwegian beavers displayed a similar paucity of genetic variation, with APD values

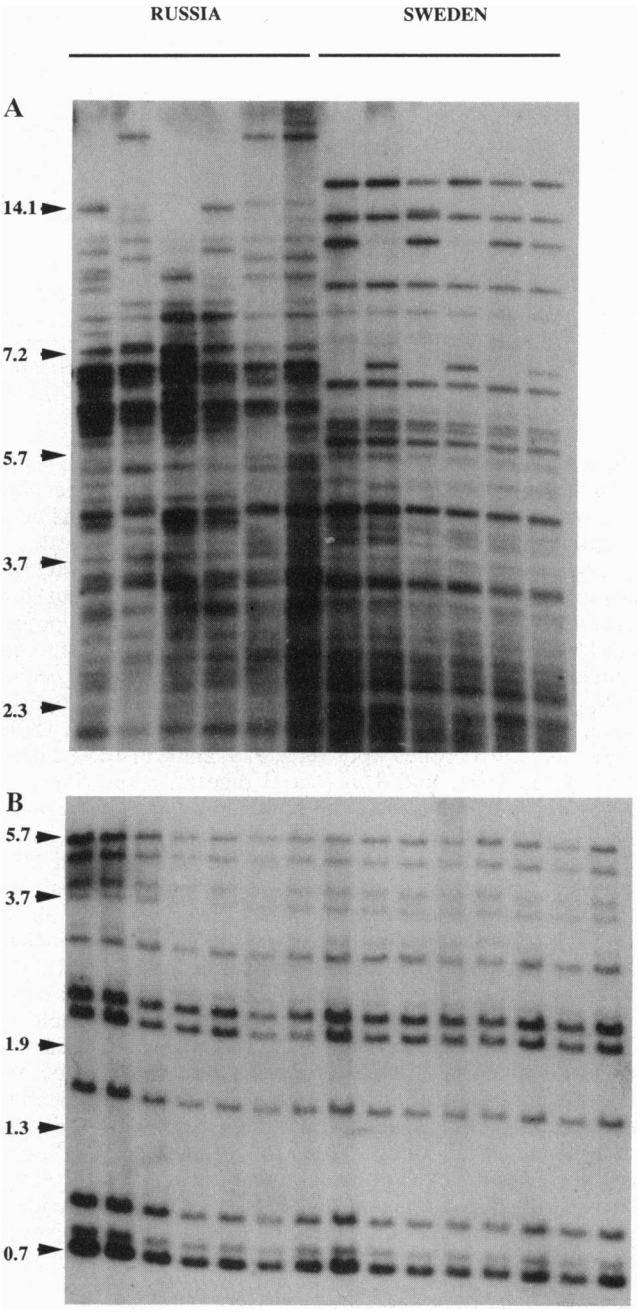

FIG. 2. (*A*) DNA fingerprints from six Swedish (right) and six Russian (left) beavers obtained with Jeffreys' 33.15 probe and *Hin*fI-digested DNA. (*B*) Southern blot analysis of 19 Swedish beaver DNAs digested with *Pvu* II and hybridized with a human MHC class II DRB probe. DNA size markers are indicated in kbp.

(16.1–20.4) close to those determined for the Swedish animals. There were no obvious differences between APD values calculated within or between countries (Table 1), as if the samples were derived from the same population. We thus conclude that the low genetic variability of Swedish animals cannot be explained by founder effects and genetic drift but, rather, is a consequence of diminished variation in the Norwegian population (predating the reintroductions to Sweden). The lack of further reduction in genetic variability in the Swedish population is compatible with the rapid population increase after the reintroductions (36).

In contrast to the Scandinavian animals, Russian beavers showed substantial DNA fingerprinting variability (APD range, 47.2–55.3; Table 1, Fig. 2*A*). We found evidence for

Table 1. APD values within and between different beaver populations as revealed by three DNA fingerprinting and MHC probes

Comparison	DNA fingerprinting probe			MHC probe	
	M13	MS6	MS15	Class I	Class II
Within population					
Sweden	22.5/23.6	15.0/10.8	16.3/13.2	0/0	0/0
Norway	20.4/16.1	11.1/6.5	11.1/6.5	0/0	0/0
Russia	55.3/54.9	47.9/49.3	47.8/47.2	0/0	0/0
Between populations					
Sweden–Norway	20.8/ND	16.2/12.0	17.6/12.7	0/0	0/0
Sweden–Russia	77.6/71.5	61.7/45.5	67.4/60.4	0/11.1	0/10.6

DNA was digested with *Hin*fI (first value) or *Hae* III (second value) when hybridized with the minisatellite probes M13, MS6, and MS15 and with *Pvu* II (first value) or *Taq* I (second value) when hybridized with MHC class I, class II DQA, DRB, DQB, or DPB probes. Data for the class II probes were pooled. ND, not determined.

differentiation between the Russian and the Scandinavian populations since inter-APD values were clearly higher than Russian intra-APD values in five of six probe/enzyme combinations (Table 1).

The degree of genetic polymorphism at beaver MHC loci was assessed by RFLP analysis using human class I and class II cDNA probes. One class I and four different class II probes (DQA, DQB, DPB, and DRB) all yielded distinct and convincing hybridization signals when probed to genomic beaver DNA digested with *Taq* I or *Pvu* II. The numbers of hybridizing fragments per individual for the class I, DQA, DQB, DRB, and DPB probes were 18, 7, 9, 9, and 8 in *Taq* I digests and 14, 5, 8, 11, and 8 in *Pvu* II digests, respectively. A tentative interpretation of the obtained restriction fragment patterns indicated that the human probes cross-hybridized to multiple class I genes, one or two DQA, DQB, and DPB genes, and at least two DRB genes. Completely identical, and thus monomorphic, patterns were observed with all 10 probe/enzyme combinations throughout 46 Swedish (including controls) and Norwegian animals (Fig. 2*B*, Table 1). The Russian beavers were also MHC monomorphic and with two exceptions identical to the Scandinavian animals (Table 1). The only differences were noted for *Taq* I digests probed with class I and class II DPB probes, where the size of one hybridizing fragment differed between Russian and Scandinavian animals (consistent with a single restriction site polymorphism at each locus). Thus, the absence of MHC polymorphism within each population and the very low diversity between the Scandinavian and Russian samples provide a strong argument for low levels of MHC polymorphism in European beavers.

DISCUSSION

Our results show that the Scandinavian beaver population is depauperate of genetic variation. Similarly, Hoppe *et al.* (37) found low levels of protein polymorphism in an expanding population of the closely related species, *Castor canadensis*, in North America; the American beaver population has also been drastically reduced in the past. The fact that Russian beavers display DNA fingerprints with substantial variability illustrates, however, that extremely low levels of genetic variation are not a general phenomenon for the European beaver. The documented bottleneck during the 19th century may explain the extreme monomorphism of Scandinavian animals, although we cannot exclude the possibility that a more ancient event has contributed to this situation. DNA typing of museum specimens by means of PCR-analyzed microsatellite markers (38) could be a useful approach to address the polymorphism status before the bottleneck.

The rapid expansion of the Scandinavian beaver population during the last 50–150 years suggests that the loss of genetic variability has not seriously affected viability or reproductive performance. This contrasts with the data on the cheetah and the lion (*Panthera leo*) in which impaired reproductive function has been explained as a consequence of inbreeding depression (5, 9). How can the apparent paradox of a rapid population growth despite a depauperate genetic variability be explained? The beaver became extinct from Sweden (and dramatically reduced in Norway) solely due to hunting at a time when pelt and castoreum from beavers had a significant economical importance. (Castoreum is an odorous substance from a pair of large glands in the region of the beaver's cloaca that is used in scent communication. In historic times castoreum was highly valued for medical purposes.) When the species was reintroduced, the hunting pressure was removed while habitats were not severely altered or occupied by any interspecific competitors. Hence, the reintroduction of the beaver was not prevented by unsuitable ecological conditions. Second, it is clear that the costs of inbreeding vary extensively between species (14, 39) and appear to be a function of the frequency of unfavorable recessive alleles in the gene pool.

It is possible that the beaver is tolerant to periods of inbreeding due to its population structure. It lives in small colonies most often composed of a single family. When subadults emigrate from the colony, dispersal is usually restricted to the natal water drainage system, since water sheds act as dispersal barriers. The probability of matings between relatives is thus likely to be higher than for many mammalian species. Indeed, beavers are known to lack strong behavioral barriers against close inbreeding and parent–progeny pair bonds have been observed in several studies (40–42). Such recurrent inbreeding events are supposed to purge the gene pool of recessive unfavorable alleles, a combined effect of genetic drift and selection against recessive homozygotes.

The present study demonstrates that the European beaver appears to be one of the few mammalian species studied so far with extremely low levels of MHC variability. It is our strong view that the methodology employed is an appropriate and reliable indicator for the degree of functional MHC polymorphism. The same human probes as used here reveal extensive genetic variation closely correlating with expressed MHC polymorphism in cattle and in chicken (43, 44). Similar observations have been made for other class I cDNA probes (34). Furthermore, the polymorphic parts of some MHC genes are surprisingly well conserved between distantly related mammals (45). Present data do not exclude the possibility that there is some functional MHC polymorphism in beavers as variability may occur in coding regions despite lack of RFLPs. However, the completely monomorphic MHC RFLP patterns strongly suggest that the degree of MHC polymorphism in beavers is very limited. This conclusion is particularly well founded for the class II genes, as we

used probes corresponding to the four most polymorphic class II genes in humans (DQA, DQB, DRB, and DPB).

Although high levels of MHC variability are the common feature of most mammalian species studied so far (15), there are a few species with documented low degrees of MHC polymorphism, such as the Syrian hamster (*Mesocricetus auratus*) (46), the African cheetah (16), and possibly also some whale species (47). For the cheetah, the lack of MHC polymorphism has been suggested to be a consequence of a previous population bottleneck. We find it unlikely that the low levels of MHC polymorphism in the European beaver could be attributed solely to population history, since MHC monomorphism was documented also in the Russian population that was polymorphic for DNA fingerprints. Rather, the observed patterns may reflect a reduced selection pressure for MHC polymorphism in this species.

The observation of MHC monomorphism among the viable Scandinavian beaver population adds an important perspective to conservation genetics since the preservation of MHC allelic diversity has been proposed to be a major goal in all conservation programs of vertebrate species (48). However, this strategy has been criticized on the basis that it puts too much emphasis on a single genetic system and may lead to the loss of genetic variability at other important loci (49, 50). Moreover, our study illustrates that there is no simple relationship between population viability and genetic diversity, the main conclusion being that the loss of genetic variability may not necessarily exclude the survival of an endangered population provided that the ecological conditions are appropriate. The Swedish beaver conservation program has been successful despite the low levels of genetic variability in general and at MHC loci in particular, in the founder population. Of course, this does not imply that genetic variability is unimportant for endangered populations, as the beaver may be unusual in its tolerance to inbreeding. Furthermore, it is an open question whether the loss of genetic diversity in the Scandinavian beaver population may influence its capability to cope with future environmental changes. However, the beaver data warn against generalized conclusions concerning the importance of genetic variability for the survival of an endangered population.

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