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# Genetic consequences of population decline in the European otter (*Lutra lutra*): an assessment of microsatellite DNA variation in Danish otters from 1883 to 1993

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The European otter (*Lutra lutra*) was common in Denmark until the 1960s, but its present distribution encompasses only a minor part of the country. The aim of this study was to assess whether the recent population decline has resulted in loss of genetic variability and to gain further insight into the dynamics of the population decline. This was done by analysing microsatellite DNA variation in contemporary and historical samples, the latter encompassing DNA samples extracted from museum specimens covering a time-span from the 1880s to the 1960s. Tests for differences in expected heterozygosity and the numbers of alleles in contemporary versus historical samples and a test for detecting population bottlenecks provided few indications of a recent bottleneck and loss of variability. However, a procedure for detecting population expansions and declines, based on the genealogical history of microsatellite alleles, suggested that a drastic long-term population decline has taken place, which could have started more than 2000 years ago, possibly due to ancient anthropogenic pressure. Finally, assignment tests and pairwise $F_{ST}$ values suggested weak but statistically significant genetic differentiation between the extant population and historical samples of otters from other regions in Denmark, more likely reflecting differentiation among original populations rather than recent drift.

**Keywords:** ancient DNA; conservation; *Lutra lutra*; microsatellites; population bottleneck; temporal variation

## 1. INTRODUCTION

Many species and populations are in danger of becoming extinct or losing significant proportions of their genetic variability (Avise & Hamrick 1996). Typically, the species that have been studied were previously abundant and distributed over large geographical areas but are now found in only a few small isolated populations (e.g. Vrijenhoek 1994; Bouzat *et al.* 1998). Fragmentation and reduced population sizes will eventually lead to loss of genetic diversity and fitness (Lande & Barrowclough 1987). However, assessment of the loss of variability that has actually taken place is often hampered by lack of information about the genetic composition of the same populations prior to the fragmentation and bottleneck. Moreover, there generally exists little knowledge about the variation that has been lost when populations have become entirely extinct. Some recent studies have solved this problem by extracting 'ancient' DNA and analysing molecular markers from museum or other types of historical samples and comparing the results with present populations (Taylor *et al.* 1994; Nielsen *et al.* 1997; Bouzat *et al.* 1998).

The European otter (*Lutra lutra*) is endangered in several regions of Europe (Macdonald & Mason 1994). It was previously distributed all over Denmark, but has severely declined in number during the last four decades. Thus, the Danish Game Bag Record, which covers the period from 1941 to 1968 (when the species became totally protected), shows a drastic decline from *ca.* 500 individuals bagged in 1941 to 100 individuals bagged in 1967 (Strandgaard & Asferg 1980). National surveys in 1984–1991 indicated that the species had almost or totally disappeared from Sealand and Funen and is now restricted to northern and western Jutland around the Limfiord catchment area (Madsen 1996) (see figure 1).

In the present study, we focused on the genetic consequences of this population decline. The census population size of otters in the Limfiord catchment area was estimated to be 200 in the 1980s (Madsen 1996), but the effective population size ($N_e$) was perhaps considerably lower (cf. Frankham 1995). Therefore, we wanted to assess whether the present Limfiord population has suffered a significant loss of genetic variability and whether it experienced a population bottleneck. In addition, we wanted to assess the genetic relationship between the surviving otter population in the Limfiord catchment area and (extinct) otters from elsewhere in Denmark. In question is whether the remaining population was part of one big population that has declined or if genetically distinct population segments have previously been present, one of which was the Limfiord population. These issues were addressed by using analysis of microsatellite

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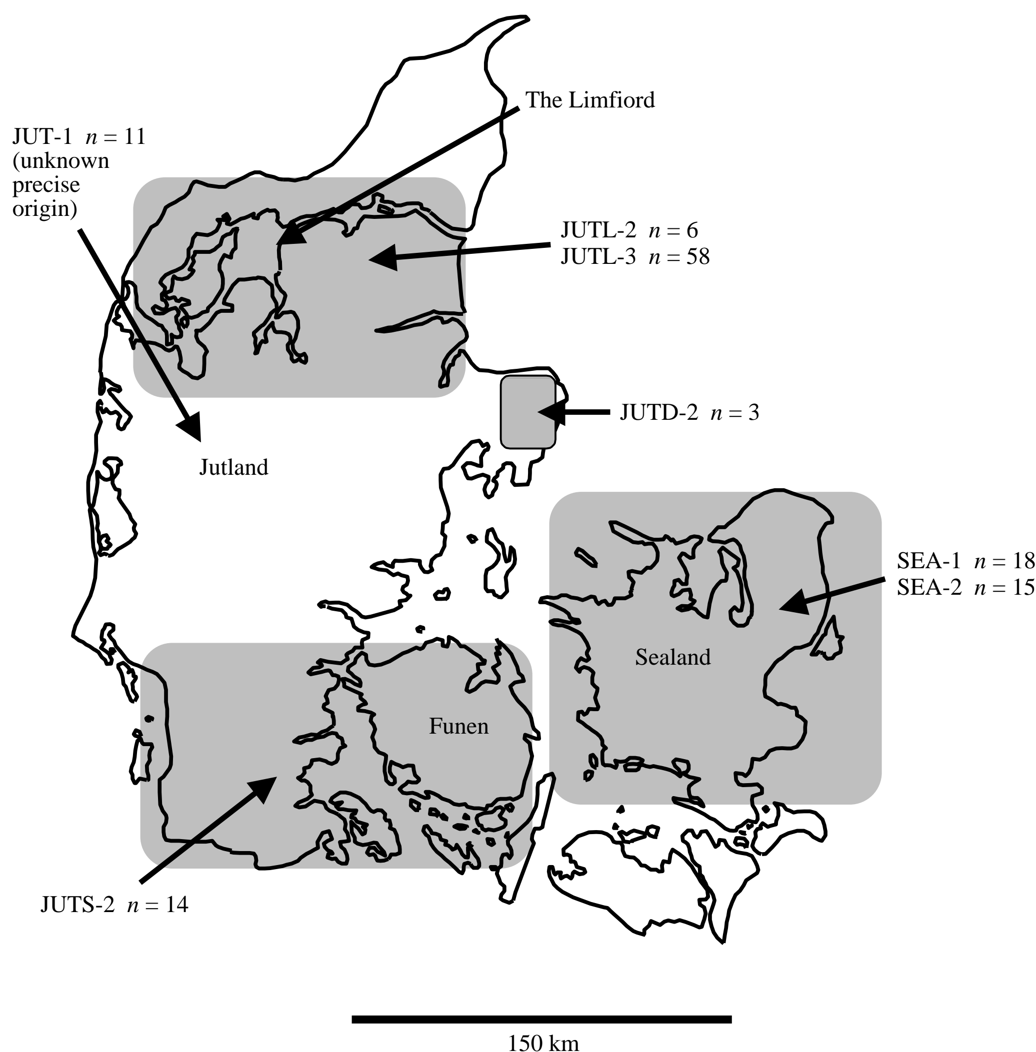


Figure 1. Map showing the approximate location of the sampling sites for the contemporary and historical samples of otters in Denmark. See § 2 for an explanation of the sample abbreviations. Time-period 1, 1883–1949; time-period 2, 1960–1963; time-period 3, 1989–1993.

DNA from museum samples of otters from Jutland, Funen and Sealand, which cover a time-span from the 1880s to the 1960s and from fresh tissue samples collected from the present otter population.

## 2. MATERIAL AND METHODS

### (a) *Samples*

The historical samples consisted of 67 otter skulls from the collections of the Zoological Museum, Copenhagen (time-period 1, 1883–1949) ($n = 32$, with eight individuals dating back before 1910) and the Natural History Museum, Aarhus (time-period 2, 1960–1963) ($n = 35$). These samples were from the islands of Funen and Sealand and the Jutland peninsula. The contemporary samples ($n = 58$), which were all from the Limfiord catchment area, consisted of frozen muscle, kidney or liver tissue samples, which were mainly obtained from road kills during the years 1989–1993 (time-period 3) (figure 1). We further subdivided the samples according to geography (see figure 1) and defined the following groups: Sealand time-period 1 (SEA-1), Sealand time-period 2 (SEA-2), various unknown sites in Jutland time-period 1 (JUT-1), southern Jutland and Funen time-period 2 (JUTS-2), eastern Jutland (Djursland) time-period 2 (JUTD-2), the Limfiord catchment area, Jutland time-period 2 (JUTL-2) and the Limfiord catchment area, Jutland time-period 3 (JUTL-3).

### (b) *Molecular analyses*

DNA was extracted from fresh tissue using standard phenol/chloroform extraction (Sambrook *et al.* 1989). The DNA from the museum specimens was extracted by removing a canine tooth from a skull and drilling out the tooth's root using a 2 mm drill. Approximately 0.1 g of tooth and tooth root were collected and DNA extraction followed the procedure of Nielsen *et al.* (1997). In order to avoid cross-contamination, the drill was heated until glowing following each collection of tooth root. In addition, no extraction of DNA from recent tissue samples was conducted in the laboratory during the time-period during which we worked with the historical samples, and polymerase chain reaction (PCR) reagents were exposed to UV radiation in a UV cross-linker in order to degrade possible contaminating DNA. Finally, PCR amplification of the fresh and historical samples was for the most part performed in separate laboratories

(the Danish Institute for Fisheries Research and the University of Aarhus, respectively).

The following microsatellite loci were assayed: Lut435, Lut457, Lut701, Lut717, Lut733, Lut782, Lut818, Lut832, Lut453, Lut833, Lut715 (Dallas & Piertney 1998) and Lut902 (Dallas *et al.* 1999). Lut453 did not yield reliable amplification and was therefore excluded from further investigations. All loci were amplified at an annealing temperature of 58 °C using 30 (contemporary samples) or 40 (historical samples) PCR cycles. The amplified loci were analysed on a Pharmacia (Uppsala, Sweden) ALFexpress automated sequencer.

### (c) *Statistical treatment*

Deviations from Hardy–Weinberg equilibrium were tested using exact tests (Guo & Thompson 1992) using the program Genepop 3.1 (Raymond & Rousset 1995*a*). In order to test equality of heterozygosity ($H_e$) in the contemporary sample of otters relative to the historical samples, all historical samples were pooled and a one-tailed *t*-test was performed based on arcsine square-root-transformed $H_e$ values. Loss of allelic variation in the contemporary sample of otters was tested by a randomization test. One thousand samples that were equal in size to the contemporary sample of otters were drawn at random from the pooled historical sample for each locus. We constructed a frequency distribution of the number of alleles in the samples generated, and assessed the probability of observing a number of alleles equal to or less than that observed in the contemporary sample of otters (for further details see Nielsen *et al.* 1999). In order to test for recent population bottlenecks, we applied the test of Cornuet & Luikart (1996) using the software Bottleneck 1.2. We performed the tests assuming an infinite allele model, a stepwise mutation model and a two-phase model of mutation (with 95% stepwise mutation models). We tested both the contemporary and historical samples, as we could not *a priori* exclude the possibility that Danish otters had experienced a bottleneck prior to the time-span covered by the samples. Finally, we used the procedure of Beaumont (1999) for detecting population declines and expansions. It assumes a stepwise mutation model and estimates the posterior probability distribution of several genealogical and demographic parameters using Markov chain Monte Carlo simulations based on the observed distribution of microsatellite alleles and their repeat numbers. The most important output parameters are (i) $r$, which is defined as $N_0/N_1$, where $N_0$ is the current effective number of chromosomes and $N_1$ is the number of chromosomes at some point back in time $t_f$ and (ii) $t_f$, which is defined as $t_a/N_0$, where $t_a$ denotes the number of generations that have elapsed since the decline or expansion began. Finally, the procedure estimates $\theta$, which is defined as $2N_0\mu$, where $\mu$ denotes the mutation rate. We performed the analyses for both the contemporary sample of otters (JUTL-3) and the pooled samples from time-period 1 assuming both linear and exponential modes of decline.

Genetic differentiation between samples was assessed using exact tests (Raymond & Rousset 1995*b*) using Genepop 3.1 and by calculating pairwise $F_{ST}$ values and testing their significance by permuting individuals between samples with the program Arlequin 1.1 (Schneider *et al.* 1997). The very small samples JUTL-2 and JUTD-2 were excluded from the analyses. Individuals' population of origin was assessed using assignment tests (Paetkau *et al.* 1995), where individuals were assigned to the sample in which they had the highest 'probability of belonging' based on their multilocus genotypes. We used the program Geneclass 1.0 (Cornuet *et al.* 1999) and chose the 'Bayesian approach', as recommended by the authors.

## 3. RESULTS

### (a) *Genetic variability and population declines*

Two of the loci were monomorphic and the nine remaining loci amplified well and were polymorphic. The success of amplification ranged between 96 and 100% among loci, even for the historical samples. Previous studies have shown that allelic drop-outs (amplification of just one of two alleles) may occur in analysis of degraded DNA obtained from old teeth and bones (Zierdt *et al.* 1996), presumably due to a scarcity of intact DNA templates (Hummel & Herrmann 1995). However, when the quality and quantity of the DNA extracted from the fresh and historical samples was compared on a 2% agarose gel, the quantity of the DNA from the historical samples did not differ much from that obtained from the fresh samples. The historical DNA samples were degraded to a greater extent than the fresh samples, but there was clearly a proportion of larger DNA fragments present ($>500$ bp). We extracted DNA from another canine tooth belonging to the same skull in six specimens where some of the loci were found to be homozygotic and observed the same genotypes (i.e. the same homozygotic alleles). Hence, the quality and quantity of the DNA extracted from the teeth of the historical samples and the reproducibility of the results leads us to assume that problems with allelic drop-outs have not significantly affected our results. Four significant deviations from Hardy–Weinberg equilibrium were observed: all were found in the contemporary sample of otters (JUTL-3) and all were due to heterozygote deficiency (see table 1). The levels of polymorphism were low with the total numbers of alleles per locus ranging from two to five in both the contemporary and historical samples (table 1). The mean number of alleles per locus was 3.9 in the pooled historical sample and 3.1 in the JUTL-3 sample. The *t*-test for equality of expected heterozygosity in the JUTL-3 sample as compared with the historical samples yielded a non-significant result ($p=0.19$). The randomization tests for reduced allelic variability in the JUTL-3 sample yielded a significant outcome for loci Lut435, Lut457 and Lut782 (all $p<0.001$). Tests for population bottlenecks did not result in significant outcomes in either the case of the JUTL-3 sample or the case of the historical samples. The procedure used for assessing population declines and expansions (Beaumont 1999) suggested that the population sizes in both time-periods 1 and 3 had declined drastically to *ca.* 1–3% of the original population size (modes of $\log(r)=-1.91$ (time-period 1) and $\log(r)=-1.555$ (time-period 3)) (table 2). Furthermore, the analysis suggested that this decline had taken place over a long time-period (modes of $\log(t_f)=0.989$ (time-period 1) and $\log(t_f)=0.98$ (time-period 3)) (Table 2). If we assume a current effective number of chromosomes of roughly $N_0=100$ and a generation time of 3 years, this suggests that the decline started *ca.* 2000–3000 years ago. These results were based on a linear model of population decline. Simulations assuming an exponential model also pointed to a drastic decline over a long time-span (table 2), but as this model is primarily

Table 1. Summary of the observed number of alleles per microsatellite locus, outcome of tests for deviations from expected Hardy–Weinberg proportions, expected ($H_e$) and observed ($H_o$) heterozygosities and sample sizes ($n$) of the populations studied.

(Table-wide significance levels were applied using the sequential Bonferroni technique (Rice 1989) (initial $k = 44$). See § 2 for an explanation of the sample abbreviations. $^{*}p < 0.05$ and $^{***}p < 0.001$. A dash indicates a test which was not performed due to small sample sizes (< 10). The total numbers of alleles for each locus are given in parentheses.)

| locus | SEA-1 | SEA-2 | JUT-1 | JUTL-2 | JUTS-2 | JUTD-2 | JUTL-3 |
|---|---|---|---|---|---|---|---|
| Lut818 (four alleles) | | | | | | | |
| number of alleles | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | n.s. |
| $H_o$ | 0.688 | 0.533 | 0.364 | 0.500 | 0.643 | 1 | 0.527 |
| $H_e$ | 0.615 | 0.508 | 0.329 | 0.439 | 0.505 | 0.733 | 0.462 |
| $n$ | 16 | 15 | 11 | 6 | 14 | 3 | 55 |
| Lut701 (three alleles) | | | | | | | |
| number of alleles | 3 | 3 | 3 | 3 | 3 | 2 | 3 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | n.s. |
| $H_o$ | 0.500 | 0.600 | 0.727 | 0.500 | 0.538 | 0.333 | 0.158 |
| $H_e$ | 0.417 | 0.476 | 0.515 | 0.439 | 0.526 | 0.333 | 0.210 |
| $n$ | 18 | 15 | 11 | 6 | 13 | 3 | 57 |
| Lut733 (five alleles) | | | | | | | |
| number of alleles | 4 | 4 | 2 | 2 | 3 | 2 | 5 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | *** |
| $H_o$ | 0.389 | 0.400 | 0.091 | 0.167 | 0.214 | 0.333 | 0.421 |
| $H_e$ | 0.456 | 0.559 | 0.455 | 0.167 | 0.204 | 0.333 | 0.636 |
| $n$ | 18 | 15 | 11 | 6 | 14 | 3 | 57 |
| Lut435 (five alleles) | | | | | | | |
| number of alleles | 5 | 4 | 1 | 2 | 3 | 2 | 3 |
| Hardy–Weinberg test | n.s. | n.s. | — | — | n.s. | — | n.s. |
| $H_o$ | 0.333 | 0.400 | 0 | 0 | 0.231 | 0.667 | 0.121 |
| $H_e$ | 0.598 | 0.453 | 0 | 0.303 | 0.335 | 0.533 | 0.203 |
| $n$ | 18 | 15 | 10 | 6 | 13 | 3 | 58 |
| Lut832 (four alleles) | | | | | | | |
| number of alleles | 4 | 4 | 3 | 2 | 4 | 2 | 4 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | * |
| $H_o$ | 0.556 | 0.467 | 0.500 | 0.800 | 0.462 | 0.333 | 0.397 |
| $H_e$ | 0.554 | 0.605 | 0.484 | 0.533 | 0.554 | 0.600 | 0.577 |
| $n$ | 18 | 15 | 10 | 5 | 13 | 3 | 58 |
| Lut717 (two alleles) | | | | | | | |
| number of alleles | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | * |
| $H_o$ | 0.556 | 0.667 | 0.545 | 0.667 | 0.571 | 0.667 | 0.610 |
| $H_e$ | 0.489 | 0.46 | 0.485 | 0.485 | 0.423 | 0.533 | 0.424 |
| $n$ | 18 | 15 | 11 | 6 | 14 | 3 | 55 |
| Lut782 (five alleles) | | | | | | | |
| number of alleles | 3 | 4 | 5 | 2 | 4 | 1 | 2 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | n.s. |
| $H_o$ | 0.333 | 0.333 | 0.545 | 0.667 | 0.286 | 0 | 0.351 |
| $H_e$ | 0.375 | 0.407 | 0.528 | 0.485 | 0.429 | 0 | 0.335 |
| $n$ | 18 | 15 | 11 | 6 | 14 | 3 | 57 |
| Lut902 (four alleles) | | | | | | | |
| number of alleles | 4 | 3 | 3 | 4 | 3 | 3 | 4 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | n.s. |
| $H_o$ | 0.600 | 0.267 | 0.545 | 0.667 | 0.429 | 1 | 0.464 |
| $H_e$ | 0.572 | 0.543 | 0.589 | 0.561 | 0.521 | 0.733 | 0.563 |
| $n$ | 15 | 15 | 11 | 6 | 14 | 3 | 56 |
| Lut457 (three alleles) | | | | | | | |
| number of alleles | 3 | 3 | 3 | 3 | 3 | 3 | 2 |
| Hardy–Weinberg test | n.s. | n.s. | n.s. | — | n.s. | — | *** |
| $H_o$ | 0.118 | 0.333 | 0.182 | 0.2 | 0.143 | 0.333 | 0.187 |
| $H_e$ | 0.348 | 0.517 | 0.385 | 0.511 | 0.265 | 0.600 | 0.416 |
| $n$ | 17 | 15 | 11 | 5 | 14 | 3 | 55 |

Table 2. Summary statistics for the otter data collected in time-periods 1 and 3.

(The lower 0.9 High Density Point (HDP) limit, mode and upper 0.9 HDP limit for three parameters analysed using the linear and exponential model declining trends. The parameter values (respectively lower and upper bounds) used for running the simulations are shown in the first column.)

| | | time-period 1 | | | time-period 3 | | |
|---|---|---|---|---|---|---|---|
| parameter | values | lower 0.9 HDP limit | mode | upper 0.9 HDP limit | lower 0.9 HDP limit | mode | upper 0.9 HDP limit |
| linear model | | | | | | | |
| $\log(r)$ | −2.3, 1.0 | −1.848 | −1.910 | −1.836 | −1.655 | −1.555 | −1.651 |
| $\log(t_f)$ | −5.0, 4.0 | 0.851 | 0.989 | 0.860 | 0.866 | 0.980 | 0.869 |
| $\log\theta$ | −2.0, 0.0 | −0.823 | −0.635 | −0.799 | −0.949 | −0.837 | −0.937 |
| exponential model | | | | | | | |
| $\log(r)$ | −2.3, 1.0 | −2.162 | −2.291 | −2.138 | −2.212 | −2.140 | −2.194 |
| $\log(t_f)$ | −5.0, 4.0 | 0.323 | 0.353 | 0.332 | 0.560 | 0.585 | 0.567 |
| $\log\theta$ | −2.0, 0.0 | −0.769 | −0.845 | −0.741 | −0.780 | −0.791 | −0.922 |

Table 3. Pairwise $F_{ST}$ values (above diagonal) and exact tests for homogeneity of the allele frequencies with probabilities combined over loci using Fisher's method (below diagonal).

(n.s., not significant.)

| | SEA-1 | SEA-2 | JUT-1 | JUTS-2 | JUTL-3 |
|---|---|---|---|---|---|
| SEA-1 | — | 0 | 0.029 | 0.004 | 0.034* |
| SEA-2 | n.s. | — | 0.022 | 0.000 | 0.023 |
| JUT-1 | n.s. | n.s. | — | 0.043 | 0.064** |
| JUTS-2 | n.s. | n.s. | n.s. | — | 0.056** |
| JUTL-3 | *** | *** | *** | *** | — |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

valid for short-term strong declines (Beaumont 1999), we emphasize the results based on a linear model.

### (b) *Genetic population structure*

The exact tests for differences in allele frequencies between samples showed significant differentiation between the JUTL-3 sample and all other samples, whereas no significant differentiation was observed between the historical samples (table 3). Similarly, the pairwise $F_{ST}$ values were low (0–0.043) and non-significant between the historical samples, but were slightly higher (0.023–0.064) and, in all but one case, significant between the JUTL-3 sample and the other samples. It is of course possible that some differentiation existed between otters from Sealand and southern Jutland, but that the sample sizes were too small for obtaining sufficient statistical power. We assessed the magnitude of this problem by randomly reducing the sample size of the JUTL-3 sample to 18 and again performing the exact tests for differences in allele frequencies. Two out of four tests involving this sample were still significant at the 5% level. We used two baseline samples for the assignment tests, i.e. the JUTL-3 sample and the pooled historical samples SEA-1, SEA-2 and JUTS-2. This pooling was justified by the observation of no significant differences between the samples and by the fact that they all represented a coherent geographical region separated from the Limfiord catchment area (figure 1). The assignment tests showed that 74 and 83%, respectively, of the individuals from the two baseline samples were assigned correctly (data not shown). The individuals from the historical sample from Jutland but of unknown precise geographical origin (JUT-1) and the three individuals from eastern Jutland (JUTD-2) were all assigned to the pooled historical sample. Conversely, four of the six individuals sampled from the Limfiord catchment area in the 1960s (JUTL-2) were assigned to the present population (JUTL-3).

## 4. DISCUSSION

### (a) *Genetic variability and population decline*

The number of alleles detected per locus (between one and five) must be considered low compared with the levels of variability that are normally reported for microsatellite loci (Goldstein & Pollock 1997). Dallas & Piertney (1998) observed higher variability at the same loci with the numbers of alleles ranging from five to 10. However, they screened individuals from a much larger area (UK, Ireland and Germany). In a more intensive study of microsatellite diversity in Scottish otters, Dallas *et al.* (1999) observed mean numbers of alleles per locus ranging from 2.1 to 5.3. The mean numbers of alleles observed in the present study (3.9 for the historical samples and 3.1 for the JUTL-3 sample) fall within this range. Other studies of otter populations from Denmark and Germany employing analyses of mitochondrial DNA have also revealed low variability (Effenberger & Suchentrunk 1999; Mucci *et al.* 1999; Cassens *et al.* 2000). Thus, it must be concluded that otter populations, at least those from northern Europe, exhibit low genetic variability. The present study suggests that the genetic variability of otters in Denmark was low even before the recent major decline in otter populations took place. This could be a result of the drastic population decline that started *ca.* 2000–3000 years ago, as suggested by the analyses using Beaumont's (1999) method. Alternatively, the low variability could be due to founder events during post-glacial recolonization (*ca.* 10 000 years ago). This explanation is supported by the data of Cassens *et al.* (2000). These authors sequenced the mitochondrial DNA

d-loop in otters from eastern Germany and Central Europe and, in most cases, found the same single dominant haplotype and a few rare, local haplotypes derived from the most common one by single point mutations.

There were a few indications of recent loss of variability in the extant otter population as compared with the historical samples. The tests for bottlenecks and differences in expected heterozygosity did not provide evidence of reduced variability. Only the tests for reduced numbers of alleles yielded three significant outcomes, but this may simply reflect the fact that the historical samples represent a much larger geographical range and, consequently, more genetically divergent population segments as compared with the contemporary sample. However, this does not imply that loss of variability in Danish otters has not taken place. First, the bottleneck test of Cornuet & Luikart (1996) can only detect severe and relatively recent reductions that have taken place within $0.2N_e$–$0.4N_e$ generations. In addition, the analysis suggested by Beaumont (1999) for detecting population declines and expansions suggested that a severe decline had in fact taken place and that the current population size had been reduced to only a few per cent of the original population size. Both the contemporary sample and the historical samples from the 1880s to the 1940s yielded qualitatively similar results, showing that the decline had taken place over a much longer time-span than that covered by the samples analysed. In fact, the analysis suggested that the decline may have started more than 2000 years ago. This is not an unreasonable estimate, given that Denmark has been relatively densely populated by humans for approximately the past 2000 years. Another riverine mammal, the European beaver (*Castor fiber*), became extinct in Denmark *ca.* 2500 years ago, presumably due to human-induced habitat destruction or hunting (Aaris-Sørensen 1988). However, as pointed out by Beaumont (1999), the results obtained by his method should also be interpreted with some caution as the analysis assumes a strict stepwise mutation model, which is probably invalid for microsatellite loci. In conclusion, the low variability in Danish otters could be explained either by post-glacial founder events or a more recent population decline, which started *ca.* 2000–3000 years ago. The two explanations are not mutually exclusive but, given the strong signal of a drastic, long-term population decline obtained by the analysis of Beaumont (1999), we assume that historical population declines have played an important role in the observed low variability in extant Danish otters.

### (b) *Genetic population structure*

The tests for genetic differences between samples indicated that the present JUTL-3 population is genetically divergent from the historical samples from southern Jutland and Sealand (table 3). The genetic divergence of the JUTL-3 sample relative to the other samples could be due to either geographical variation or shifts in the allelic frequencies in the JUTL-3 sample that were caused by genetic drift during the recent population decline. However, as discussed previously, there were few if any indications of a recent population bottleneck in the JUTL-3 sample. Furthermore, the assignment tests showed that individuals from the historical samples of unknown precise origin in Jutland (JUT-1) and from eastern Jutland (JUTD-2) were all assigned to the pooled historical baseline sample. In contrast, four of the six individuals from the historical sample from the Limfiord catchment area (JUTL-2) were assigned to the present Limfiord population, suggesting that genetic differentiation was also present in the past. The deviations from Hardy–Weinberg equilibrium observed in the JUTL-3 sample suggest that, even within this region, the otter population may not be homogenous (table 1) and that there may be some sort of population structure at an even finer geographical scale. This could also be the case in some of the other regions, where the sample sizes may have been too small for detecting deviations from Hardy–Weinberg equilibrium.

### (c) *Conclusions*

In conclusion, our study shows that canine teeth are reliable sources of historical DNA samples. As skull collections are often maintained in high numbers at museums, analysis of DNA from canine teeth may be a way of obtaining sufficient sample sizes, a problem otherwise hampering studies based on analysis of DNA from historical samples (e.g. Nielsen *et al.* 1999). Even though the historical sample sizes in the present study were higher than in previous studies, for example Taylor *et al.* (1994) ($n = 5$) and Bouzat *et al.* (1998) ($n = 15$) the design of this study was limited by the availability of samples, which have reduced the power of several statistical tests (e.g. Hardy–Weinberg equilibrium tests). A direct comparison of contemporary and historical genetic variation in the extant Limfiord population would have been preferable, but too few samples were available for that purpose. Nevertheless, despite these limitations the data presented here indicate that the extant otter population has not suffered a recent severe loss of genetic variability and that some geographical variation was present in the past. There were indications that a drastic population decline had taken place, but that this had happened on a time-scale covering hundreds or thousands of years. This suggests that the recent population decline in otters only represents an acceleration of a negative development that may date back even into prehistoric times.

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