EFFECTS OF HABITAT FRAGMENTATION ON THE EURASIAN BADGER (*MELES MELES*) SUBPOPULATIONS IN DENMARK

CINO PERTOLDI*, VOLKER LOESCHCKE*, AKSEL BO MADSEN**, ETTORE RANDI° AND NADIA MUCCI°.

* *Department of Ecology and Genetics, University of Aarhus, Building 540, Ny Munkegade, DK-8000 Aarhus C, Denmark; Cino.Pertoldi@biology.aau.dk*
** *Department of Landscape Ecology, National Environmental Research Institute, Kalø Grendvej 14, DK-8410 Rønde, Denmark*
° *Istituto Nazionale per la Fauna Selvatica, via Ca' Fornacetta 9, 40064 Ozzano Emilia (Bo), Italy*

ABSTRACT - Genetic variation in five populations of the Eurasian badger from Denmark was screened, using the hyper-variable minisatellite DNA probe 33.15. Very low genetic variability was found within populations. This lack of variability could be related to the fragmentation of the Danish landscape which reduces the effective population size of local populations and the gene flow between different subpopulations. The present paper discusses the possibility of managing the Danish badger subpopulations as a metapopulation.

Key words: Meles meles, minisatellites, landscape fragmentation, Denmark.

INTRODUCTION

The Eurasian badger, *Meles meles* (Mustelidae) has a limited dispersal (Kruuk and Parish, 1982; Cheeseman *et al.,* 1987), and forms highly stable social groups (Kruuk 1978). Badger populations in Denmark, estimated to consist altogether of about 25,000 individuals, are thought to be declining (Sørensen, 1995). Denmark, with few local exceptions, shows a relatively low density of badgers (Sørensen, 1995; Taastrøm, 1993). Furthermore, Cheeseman *et al.* (1988) found that dispersal rates are low in rural areas similar to the type found in Denmark.

Badgers suffer supposedly because of habitat fragmentation as they need different habitats that include water, wood and pasture land. Moreover, human disturbance and outdoor activities have increased considerably in the last few decades together with an increased number of stray dogs that may cause olfactive stress. Also road traffic has become a ma-

jor threat. From 1983 to 1991 road traffic, measured by the number of kilometres driven on the roads, increased by 38% in Denmark (Sørensen, 1995). Killings in traffic accidents have sharply increased in the last 20 years (Sørensen, 1995).

The fact the genetic structure of natural populations of badgers may be disrupted by isolation, is a question of concern for their conservation. Small and isolated populations have a high risk of extinction and may suffer from the fixation of deleterious genes (Soulé, 1987 and references therein). The consequences of isolation and small population size include inbreeding depression and loss of genetic variation. Inbreeding is usually deleterious in species that normally outbreed, whereas when inbreeding is part of the natural social system of a species, inbreeding depression is far less severe, and the genetic load is usually low (Soulé, 1987 and references therein). The aim of this paper is to screen the genet-
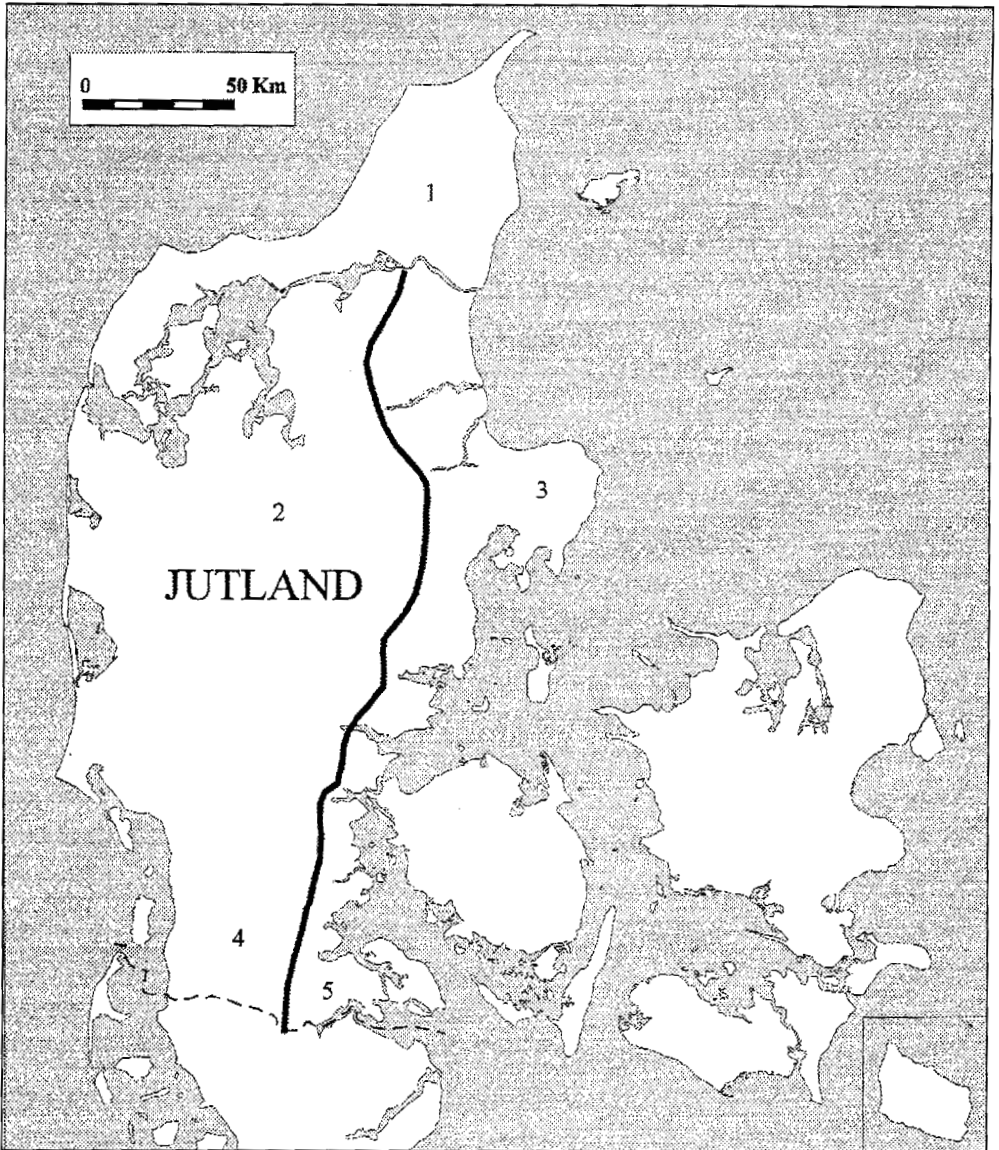
Figure 1 - Map of Denmark with the five zones of collection of badgers (full line= motorway).

ic structure of the Danish badger populations examined in populations from five different areas (with a maximum distance of 40 km between individuals, within the same zone) in Denmark (Fig. 1). The nuclear genomes were screened using the DNA fingerprint (Jeffreys et *al.*, 1985a,b; Jeffreys *et al.*, 1988) which has proven to be a powerful technique in the study of the genetic structure of pop-

ulations (Smith and Wayne, 1996 and references therein).

MATERIALS AND METHODS

Fifteen male badger bodies were collected with reports on the exact place of death, and which could be mapped within $10km^2$. Samples of muscle and liver were stored in freezers at $-30°C$ and thereafter preserved in 100%

ethanol. Genomic DNA was extracted from tissue following standard procedures including proteinase K, phenol-chloroform, chloroform-isoamyl alcohol extraction (Sambrook *et al.*, 1989). DNA samples were run on a test gel to check for degradation and to adjust concentrations. Ten micrograms of genomic DNA were restricted with an excess of enzyme Alu I overnight at 37°C. A second gel was run to test for completeness of digestion and for even concentration of samples. Electrophoresis was performed in 1% agarose gels and TBE buffer. Gels were run at 25 V for 27-28 hours.

The banding pattern obtained with Jeffreys' probe 33.6 (Jeffreys *et al.,* 1988) were light and confused and therefore could not be used for our investigation. Only the probe 33.15 gave interpretable results. Individuals showing band deviations of 1 mm were considered shared. Comparisons between individuals on different gels were made by photocopying one autoradiograph over the other.

The similarity coefficient (S_{xy}) between each pair of individuals (x and y) was calculated as the number of common bands in their fingerprint profiles (n_{xy}) divided by the average number of bands in their fingerprint profiles: $S_{xy}= 2n_{xy}/(n_x+n_y)$. The average number of bands can be used for estimating the degree of inbreeding (Lynch, 1991). Therefore the mean number of bands in the five populations was compared by an analysis of variance.

RESULTS

We found low genetic differences within populations, indicating that populations were genetically rather homogeneous (Table 1). The same banding pattern was, however, never found in two different populations (Table 1). The genetic similarity coefficient (S_{xy}) was

Table 1 - Banding pattern of the badgers collected in the five different populations (Pop.).

Individual	Pop. 1			Pop. 2			Pop. 3					Pop. 4		Pop. 5	
	1	2	3	1	2	3	1	2	3	4	5	1	2	1	2
	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1
	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	0	0	0	0	1	1	0	1	0
	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0
	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1

Table **2** - Average genetic similarity D±SD of the badger between individuals within the five different populations.

Zone	n	±SD
1	3	1±0
2	3	0.884±0.004
3	5	0.975±0.03
4	2	0.947
5	2	0.875

Table 3 - Comparison of average number of bands between subpopulations.

Zone	n. of individuals	Average n. of bands ±SD	Source of variation	df	MS	P
1	3	18±0	Between	4	4.04	0.0006
2	3	17.667±1.155	Within	10	0.32	
3	5	16±0				
4	2	19				
5	2	17.5				

different in the different populations, suggesting a higher level of inbreeding and genetic isolation (lower gene flow between the other populations) in zone 1 and 3 (Table 2). The mean number of bands was 17.3±1.2, the maximum number of bands was 19 and the minimum 16, and only six bands were shared by all individuals. The differences between the number of bands in the different populations were significant (Table 3).

DISCUSSION

The low genetic variability found in the Danish badgers makes DNA fingerprinting unsuitable for paternity testing, especially in those zones (populations 1 and 3) where the banding patterns were all monomorphic or very near to being monomorphic. In order to avoid close inbreeding, the adult badger spontaneously transfers, sometimes permanently, between adjacent social groups and mating occurs between males of one group and females of another, but habitat fragmentation nevertheless reduces this dispersal and increases the genetic heterogeneity between the different subpopulations. Therefore, we can expect close inbreeding in the isolated patches and the obtained results (the extremely low genetic variability within the single subpopulation) confirmed this hypothesis.

The low genetic variability found in population 1 was an expected result, because the region is totally isolated by the Limfjord. The low genetic variability found in population 3 (population 3 is confined by the sea to the north, east and south) indicates a high degree of isolation from the other zones. This could be due to main roads and now motonvays (with rather high traffic intensity), west of the population which separates the population living in zone 3 from the nearest population living in zone 2. The genetic difference found between the adjacent populations 4 and 5 could also indicate a degree of isolation due to the highway that divides the two populations. However, the higher genetic similarity found in zone 4 compared to that found in zone 5 could suggest a higher degree of isolation in zone 4. That could be the consequence of a prevalent agricultural landscape that is thought

to reduce the migration rate and density of badgers. The extremely high genetic similarity that was found within the single populations, is consistent with evidence of inbreeding and limited dispersal obtained from field studies of ecology and behaviour of badgers. The fact that within populations genetic variability was low, and that the same banding pattern was never found in two populations together, could indicate a geographic partitioning, which can reveal some fragmentation effect, like the increased genetic difference between subpopulations. The different average number of bands found in the different populations could also indicate that the populations differ in effective population size (N_e). The road killings may also play an important role in reducing the genetic variability, because it reduces the effective population size (N,) by about 10% (Sørensen, 1995). Dutch findings indicate that the loss of badgers to road traffic is particularly significant, and these sources of mortality equal the annual level of cub production (Griffiths *et al.*, 1993). Therefore, fauna passages should be built in zones where the gene flow is interrupted or reduced. However, the fauna-tunnels built to prevent animals from getting killed on the road have not worked for badgers (see Madsen, 1996, for review).

The preliminary results suggest (also if strong conclusions cannot be drawn because of the small sample size) that the Danish badger can be managed as a metapopulation with a gene flow of different intensities between the subpopulations living in each patch. In the metapopulation, we have local extinction and local recolonization, therefore population parameters of the Danish badger could be very useful in predicting the extinction risk in the different zones and for calculating the impact that road killings have on its population structure.

REFERENCES

Cheeseman, C.L., Cresswell, W.J., Harris, S. and Mallinson, P.J., 1988. Comparisons of dispersal and other movements in two badger *(Meles meles)* populations. Mammal Rev., 18: 51-59.

Cheeseman, C.L., Wilesmith, J.W., Ryan, I. and Mallinson, P.J., 1987. Badger population dynamics in a high-density area. Symp. Zool. Soc. Lond., 58: 279-294.

Griffiths, H.I., Griffiths, C.A. and Thomas, D.H., 1993. The Badger *(Meles meles)* An Assessment of the Population Status, Conservation Needs and Management Requirements of the Species in the Western Palaearctic. A report to the Standing Committee of the Convention on the Conservation of European Wildlife and Natural Habitats. (Convention on the Conservation of European Wildlife and Natural Habitats).

Jeffreys, A.J., Royle, N.J., Wilson, V. and Wong, Z., 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature, 332: 278-281.

Jeffreys, A.J., Wilson, V. and Thein, S.L., 1985a. Hypervariable "minisatellite" regions in human DNA. Nature, 314: 67-73.

Jeffreys, A.J., Wilson, V. and Thein, S.L., 1985. Individual-specific "fingerprints" of human DNA. Nature, 316: 76-79.

Kruuk, H., 1978. Spatial organisation and territorial behaviour of the European badger *(Meles meles).* J. Zool., 184: 1-19.

Kruuk, H. and Parish, T., 1982. Factors affecting population density, group size and territory size of the European badger, *(Meles meles).* J. Zool., 196: 31-39.

Lynch, M., 1991. Analysis of population genetics structure by DNA fingerprinting. In: Burke, T., Dolf, G., Jeffreys, A.J. and Wolff, R. (eds.), DNA Fingerprinting Approaches and Applications. Birkhauser

Verlag, Basel, pp.: 113-126.

Madsen, A.B., 1996. Odderens *(Lutru lutra)* økologi og forvaltning i Danmark. The Ecology and Conservation of the Otter (*Lutra lutra*) in Denmark. PhD Thesis. Danish Nat. Env. Res. Inst.: 84 pp.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning: a laboratory manual. 2nd edn. New York, Cold Spring Harbor Laboratory Press.

Smith, T.B. and Wayne, R.K., 1996. Molecular Genetic Approaches in Conservation. (Smith, B.T. and Wayne, R.K., eds.) Oxford University Press, Oxford.

Soulé, M.E., 1987. Viable populations for conservation. Cambridge University Press, Cambridge.

Sørensen, J.A., 1995. Road-kills of badgers *(Meles meles)* in Denmark. Ann. Zool. Fenn., 32: 31-36.

Taastrøm, H., 1993. Bestandsvurdering, home-range og gruppestørrelse hos gravlinger *(Meles meles)* i et udvalgt område, samt diskussion af problemer med gravlinger i kunstgrave. M. Sci. thesis, University of Århus.