*Conservation Genetics* **4:** 441–451, 2003.



# Complete loss of MHC genetic diversity in the Common Hamster (*Cricetus cricetus*) population in The Netherlands. Consequences for conservation strategies

M.J.M. Smulders*, L.B. Snoek, G. Booy & B. Vosman
*Plant Research International, P.O. Box 16, Nl-6700 AA Wageningen, The Netherlands (*Corresponding author: Phone: +31 317 476982; Fax: +31 317 418094; E-mail: rene.smulders@wur.nl)*

Received 22 July 2002; accepted 30 September 2002

*Key words:* Cytochrome b, DRB, haplotype, inbreeding, major histocompatibility locus, MHC, SNP, subspecies

**Abstract**

The Common Hamster (*Cricetus cricetus* L.) has suffered from changes in agricultural practices. In some Western European countries the populations have become so small and scattered that they are threatened with extinction. We studied the genetic diversity of mitochondrial and major histoincompatibility complex (MHC) loci in the few animals left in the South of the Netherlands and in three animals from the Alsace region in France, and compared it to the diversity in Dutch animals in the past (samples taken from stuffed animals in museum collections dating back to the period 1924–1956) and in a large present-day population from Czech Republic. For the mitochondrial cytochrome b gene, SNP mapping demonstrated a total of nine alleles among 14 Czech samples, of which one (possibly two) was present in the Dutch museum samples, and only one in the current Dutch animals. For the MHC genes, DQA exon 2 and 3 showed no variation, while 14 different alleles were found at DRB exon 2. The Czech population contained 13 different alleles in 15 animals sampled, and most animals were heterozygous ($H_o$ = 0.80, $H_e$ = 0.91). Therefore, the solitary living Hamster maintains, in nature, a large diversity at this MHC locus. The Dutch museum samples contained eight different alleles in 20 samples, and they were slightly less heterozygous ($H_o$ = 0.60, $H_e$ = 0.75). All but one of these alleles were also found in the Czech samples. In contrast, the present Dutch and French animals (a total of 16 samples) contained only one of these alleles, and all animals were genetically identical and homozygous. We conclude that the remaining animals have lost all diversity at this MHC locus. This is probably the result of a severe bottleneck, which may have been quite severe, reducing diversity in many loci. In addition, the remaining Dutch animals are partly derived from one family. These animals are now part of a breeding program. Options for restocking the genetic diversity are discussed.

## Introduction

The Common Hamster (*Cricetus cricetus* L.), also referred to as European Hamster or Black Bellied Hamster, originates from the lowlands of central and Eastern Europe and Siberia, living on steppes, agricultural land and river banks. Its living area extends until the Yenessey river and Altai mountains, and the Chinese province of Sinkiang (Zhang 1997). In the past, the species has thrived thanks to the expansion of agriculture, and in Eastern Europe it was (and sometimes still is) considered a pest. The last decades, however, changes in agricultural practices and disappearance of habitats have dramatically reduced the populations of the common Hamster in Western Europe, and currently the Hamster is threatened with extinction in The Netherlands, Germany, Belgium and France. Further information on its ecology may be found in Stubbe and Stubbe (1998). The Hamster is listed in Annex 2 of the Bern convention (http://www.nature.coe.int/english/cadres/bern.htm), making it obligatory to protect the species and its habitat. In the Netherlands, a species protection plan has been developed (Krekels 1999), consisting of a number

of actions, including a program for protection and restoration of habitat. It also includes a breeding program, which has been set up because the populations of Hamsters may already be too small to be self-sustainable. Estimates of the number of animals left range from several hundreds to considerably less than hundred scattered in small isolated populations in the southeastern part of The Netherlands. The number of animals caught and available for the start of a breeding program was only a few dozens (http://www.korenwolf.nl/frameset.html).

The aim of our study was to answer two questions that are, from a genetic point of view, important in such a breeding program: do the Dutch animals belong to a separate subspecies, and did they experience a severe loss of genetic diversity?

In 1899, Nehring suggested that the Hamsters in the Western European countries (in the area to the west of the river Rhine) form a different subspecies, the 'Western Hamster' *C. cricetus canescens* Nehring. He did this on the basis of a few morphological characters (more colour spots, smaller body size, slightly different cranial shape) of a very limited number of animals caught along the river Maas in Belgium. The matter has been under discussion for a century. Husson (1959) supported the subspecies status. Niethammer (1982) and Grulich (1987) rejected it. They argue that the frequency of white spots gradually increases, while body size decreases from East (Saxony) to West, with animals from the region Rhine-Hessen taking an intermediate position. Also the cranial shape changes gradually. Therefore, the Belgian and Dutch animals should be considered the end of a cline. The taxonomic status of the species has implications for conservation strategies, since the general idea is that one should not mix subspecies in breeding programs until absolutely necessary (Ryder 1986). Indeed, such was noted in the protection plan for the Hamster in the Netherlands (Krekels 1999).

Unfortunately, the delineation of subspecies is not very straightforward, but molecular information may be helpful. Molecular studies of inter- and intra-specific evolution of animals often include the mitochondrial genome (Passbøl and Arctander 1998) because of its compact size (15000–17000 nucleotides), relatively conserved gene order, absence of recombination, uni-parental inheritance, and few insertion/deletion/duplication events. The individual genes in the mitochondrial genome evolve at different rates, and the relatively rapidly evolving cytochrome b is the marker of choice in the study of "moderately diverged" interspecific relationships (Passbøl and Arctander 1998). It has been studied in a number of rodent species and subspecies (Kocher et al. 1989; Wayne et al. 1990; Wayne and Jenks 1991; Conroy and Cook 2000; Ditchfield 2000; Freeland et al. 2000; Suzuki et al. 2000), including *Mesocricetus auratus* (Golden Hamster). Here, we will determine allelic diversity in the Common Hamster by SNP mapping. If alleles are found exclusively in specific populations, and if within-taxon variation is low relative to among-taxon variation, then the argument for separate subspecies becomes stronger (Wallis 1994). In this way, clarification of taxonomy may contribute to prioritisation of conservation efforts (Haig 1998).

The same morphological signs (white spots on the belly of some animals, Kayser and Stubbe 2000) that were sometime used as evidence for a sub-species status, may also be taken as to indicate that the remaining Hamsters are the result of inbreeding. A study using microsatellite markers also indicates that the remaining Dutch Hamsters have a high level of homozygosity (Neumann et al., in prep.). Inbreeding decreases the genetic variation and the chances of survival of the population in the long term through loss of genetic adaptability and vitality (Booy et al. 2000). One genetic system of which it is generally believed that a high level of genetic diversity is beneficial, is the MHC. The genes in the MHC are thought to be of important adaptive significance, particularly for resistance to parasites and pathogens, and perhaps for mate choice and kin recognition (Klein 1986; Hill et al. 1991; Potts and Wakeland 1993; Brown and Eklund 1994; Hedrick 1994; Edwards and Potts 1996; Potts and Penn 1998). Most natural mammal populations studied possess abundant MHC diversity: high level of heterozygosity, many alleles, much sequence variation among alleles (Klein 1986; Potts and Wakeland 1990; Hedrick 1994; Hughes 1991). Although vital species and populations are known with little or no variation in MHC genes, these are usually solitary living species or populations that recently went through a "bottleneck". The Hamster lives solitary, and whether it naturally has a high level of MHC diversity will have to be assessed in a large population.

There are two classes of MHC molecules. McGuire et al. 1985 suggested that Hamster species have little or no variation between class I genes (which present intracellular peptides on the outside of the cell to T-lymphocytes). The class II genes DQA and DRB (which present extracellular proteins to T-lymphocytes) are frequently used to analyse the

genetic diversity within and among populations, e.g. in moose (Mikko and Andersson 1995; Ellegren et al. 1996) and buffalo (Wenink et al. 1998). Pfau et al. (2001) found extensive polymorphism in DQA in the rodent *Sigmodon hispidus*. We chose to use DQA and DRB to compare the diversity in present-day Hamsters with unthreatened populations.

In this study, the animals of the remaining Dutch common Hamster population (including animals now part of a breeding program) as well as a few samples from the threatened French population in Alsace were included as representatives of the putative subspecies West of the Rhine. They were compared to a large population from the Czech Republic, where the animal is still abundant. Using museum specimens, we also studied a sample of the population in The Netherlands in the period 1924–1956, before the major decrease of population size.

## Materials and methods

### *Samples*

DNA was isolated from three French hair samples using the 5% Chelex 100 protocol (Walsh et al. 1991). All other DNA samples were a gift from Alterra, Wageningen, The Netherlands (13 current Dutch samples, 20 museum animals), and Martin-Luther University, Halle, Germany (15 Czech animals in the surroundings of Brno, Czech Republic, Neumann et al., in preparation). The current Dutch animals originated from one area near Heer, and are thought to be partly from one litter. The museum animals were originally caught near Houthem (8 animals in the period 1924–1928), Oud-Valkenburg (4 animals in 1938), Heerderberg (1 animal in 1947) and Heer (3 animals in the period 1954–1956). The current Dutch animals and the museum animals are essentially from the same area in the South of the Netherlands, directly east of Maastricht (approximately 50.5° North, 5.5° East). The largest distance between sampling sites is approx. 8 km. Exact population sizes of these years are not known, but around 1900 the community of Schimmert, 4 km North of these sampling sites, paid a fee for every animal caught (Dupond 1932).

### *Primers*

Database sequences for cytochrome b, DQA and DRB (Sanjayan et al. 1996) were aligned in Megalign 5.00 (DNAStar, Madison, Wisconsin, USA) and conserved regions at the 5′ and 3′ of the exons of interest were selected for primer design using Primerselect 5.00 (DNAstar).

Cytochrome b was aligned using sequences from rat, mouse and Hamster species (AB033713, AB033702, J01436: bp312-1454, AB033698, AB033700, AB033697, AB033693, AF119265, AF119266, and AF119280). Primers were designed as to amplify overlapping sequences of around 430bp each. Primer sequences: Part 1: Forward: 5′-ATGACAATCATACGCAAAAAACACC-3′; Reverse: 5′-AATGATTTGGCCTCATGGTAG-3′. Part 2: Forward: 5′-CAGTAATAGCAACAGCATTTATAGG-3′; Reverse: 5′-TCGGGGTCTCCTAAAATATCTG-3′. Part 3: Forward: 5′-TTCATGCTATTAATTGCTCTCATAA-3′; Reverse: 5′-CAATTATGCTAGCGATTGCTATAA-3′.

The primers used to amplify DQA exon 2 and 3 were designed by aligning sequences from mouse, rat, dog and other species (M21931: bp109-639; M11356: bp17-547; M11358: bp17-547, K01925: bp1-511, X14879: bp120-805, M22366: bp370-1518, U47857: bp95-344, M30117: bp356-1334, M33304: bp359-1366, and M17236: bp413-1388). This resulted in the following primers: Target: DQA exon2; Forward: 5′-CCGACCACGTAG$^{G}/_{C}$C$^{G}/_{T}$$^{T}/_{C}$CTAT-3′; Reverse: 5′-GCTGAGTACATACTGTTGAC-3′. Target: DQA exon3; Forward: 5′-GCGAC$^{T}/_{C}$GTGTT$^{C}/_{T}$$^{C}/_{T}$CCAAGTC-3′; Reverse: 5′-TCCAGGCCCCAGTGCTCCAC-3′. Primers for the amplification of DRB exon 2 were designed using an alignment of a comparable series of sequences (M77153: bp101-370; M77154: bp101-370, M11161: bp101-370, M29611: bp140-409, M12382: bp38-307, K01145: bp15-284, U77067: bp152-421, M93432: bp108-377, and M55165: bp137-406). Primer sequences for DRB exon2: Forward: 5′-GAGTGTCATTTCTACAACGGGA-3′; Reverse: 5′-CTCTCCGCGGCACAAAGGAA-3′.

### *Amplification*

The reaction mix used consisted of 2 or 2.5 mM $MgCl_2$, 1 mM of each dNTP, 10 pmol of forward and reverse primer, 0.5 U Goldstar Taq polymerase (Invitrogen, Breda, The Netherlands), 75 mM Tris HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20 and 5–50 ng DNA. Since the quality and amount of DNA varied among samples, several PCR programs were used on a MJ Research PTC-200 (Biozym, Landgraaf, the Netherlands) thermocycler: (i) for samples with a

DNA concentration of at least 50 ng/ul: 3 min 96 °C, 30 cycles of (1 min 96 °C; 1 min 50 °C; 1 min 72 °C), with 2 mM $MgCl_2$; (ii) for samples containing 10–50 ng/$\mu$l DNA: 3 min 96 °C, 40 cycles of (30 s 96 °C; 30 s 50 °C; 45 s 72 °C), including 2.5 mM $MgCl_2$ in the reaction mixture; (iii) for samples with a very low DNA concentration (<10 ng/$\mu$l DNA) 5 min 96 °C, 10× (1.5 min 96 °C; 1.5 min 50 °C; 1.5 min 72 °C) followed by 30 cycles (30 sec 96 °C; 30 sec 50 °C; 45 sec 72 °C) at 2.5 mM $MgCl_2$.

*Sequencing*

Electrophoresis of 10 ul of the amplified mixture was done in a 3% agarose gel (Invitrogen) in 1 × TBE-buffer (12.1 g/l Tris-HCl, 6.8 g/l Boric acid, 2.5 mM EDTA) and the DNA was stained with ethidium bromide (10 mg/l). The gel fragment containing the amplified product was excised from the gel. The DNA was isolated, by spinning through a QiaQuick column (Qiagen) without using extra isopropanol. The isolated fragments were sequenced in a 20 $\mu$l reaction mix containing 5 $\mu$l template (50–300 ng DNA), 4 $\mu$l Big-dye, 4 $\mu$l sequencing buffer, 5 pmol primer, for 24 cycles (96 °C 10 s; 50 °C 5 s; 60 °C 4 min; all ramps at 1 °C/s). The fragments were purified by 5 min spinning through a Sephadex-G50 column, and sequenced on a ABIprism 3700 automatic sequencer (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Both forward and reverse strands were sequenced.

*Sequence analysis*

The sequences (traces) where manually corrected with Seqman 5.00 (DNAStar) by comparing the forward and reverse strands. The three separate parts for the cytochrome b gene were assembled into one contig. Whenever there appeared to be double peaks in the sequence traces of the DRB fragments, these were subcloned and sequenced. For this, the fragments were eluted from gel, purified, ligated into the multiple cloning site of the TOPO TA vector and transformed into a competent *E. coli* strain (TOPO One Shot cloning kit; Invitrogen) according to the manufacturers' specifications. Transformants were selected on solid LB medium containing 100 mg/L ampicilline after overnight growth at 37 °C. Up to eight colonies where picked randomly for colony PCR, which was done by incubating part of the colony in the PCR reaction mix for 5 min at 96 °C followed by PCR program (ii). For all samples that are reported to be heterozygous, the two different alleles in one animal were positively confirmed in this way. Eleven of the 14 different DRB alleles were found in more than one animal. The other three alleles were found in only one Czech animal each. The exact sequence of these alleles was obtained in at least three clones each, which indicates that no artefacts as a result of the PCR amplification steps are present.

SNPs were mapped by aligning the corrected sequences with the computer program Megalign 5.00 (DNAStar). All cytochrome b and DRB alleles differed from each other at at least two positions. A similarity score was calculated from a pairwise alignment of all possible pairs of sequences (Wilbur and Lipman 1983), and this distance matrix was used to generate a UPGMA tree with the Clustal V algorithm (Higgins and Sharp 1989), using the default values in Megalign. Expected heterozygoty and $F_{st}$ were calculated using TFPGA (Miller 1998). EMBL accession numbers of the sequences are listed in Tables 2 and 3.

## Results

*Cytochrome b variation*

The sequences of cytochrome b alleles of the Common Hamster were most similar to the Golden Hamster (*Mesocricetus auratus* AF119265) and Chinese Hamster (*Cricetulus griseus* AB033693) equivalents (81.0–82.0 and 83.8–84.3% on DNA level, 93.3-93.6 and 94.1–94.6% on amino acid level, respectively). The museum animals contained the same allele as found in samples from living animals (see below), indicating that the sequences are derived from Hamster DNA and not from contaminations during DNA extraction.

Among the Hamster samples, 20 SNPs were detected in a total of 1117 bp, giving rise to nine different alleles (Table 1). Compared to the cytochrome b sequences from related Cricetinae species as well as other rodent species (Figure 1), the number of variable sites among the Hamster alleles is relatively small. It is, e.g., much smaller than the variation between the two *Mus musculus* subspecies (*M. musculus musculus* and *M. musculus domesticus*). On the other hand, it is larger than the difference between *Phodopus campbelli* and *P. sungorus*.

The current Dutch population contained only one allele (Crcr-CytB*1). In the Dutch museum samples, amplification of the cytochrome b gene was difficult,

*Table 1.* SNPs found in the CytB-gene

| Haplotype | Nucleotide position | | | | | | | | | | | | | | | | | | | | Number of times found | | | EMBL accession |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 28 | 67[a] | 78 | 117 | 135 | 165 | 201 | 219 | 357 | 490[b] | 732 | 819 | 873 | 927 | 942 | 1000 | 1050 | 1053 | 1059 | 1071 | NL | M | CZ | number |
| Crcr-CytB*1 | C | G | C | T | C | C | C | T | C | G | A | C | A | A | C | T | T | T | C | A | 12 | 3[c] | 1 | AJ490302 |
| Crcr-CytB*2 | C | G | C | T | C | C | T | T | C | A | A | C | A | A | C | T | T | T | C | A | 0 | 0[c] | 1 | AJ490303 |
| Crcr-CytB*3 | C | G | C | T | C | C | C | T | C | A | G | T | G | G | C | C | T | A | T | G | 0 | 0 | 1 | AJ490304 |
| Crcr-CytB*4 | C | G | C | T | C | C | C | T | C | A | G | T | G | G | T | C | T | A | T | G | 0 | 0 | 1 | AJ490305 |
| Crcr-CytB*5 | C | G | T | C | T | T | T | A | T | A | G | T | G | G | C | C | T | A | T | G | 0 | 0 | 2 | AJ490306 |
| Crcr-CytB*6 | T | G | T | C | T | T | T | A | T | A | G | T | G | G | C | C | T | A | T | G | 0 | 0 | 2 | AJ490307 |
| Crcr-CytB*7 | T | G | T | C | T | T | T | A | T | A | G | T | G | G | T | C | T | A | T | G | 0 | 0 | 2 | AJ490308 |
| Crcr-CytB*8 | T | G | C | T | T | T | T | A | T | A | G | T | G | G | C | C | T | A | T | G | 0 | 0 | 2 | AJ490309 |
| Crcr-CytB*9 | T | A | T | C | T | T | T | A | T | A | G | T | G | G | C | C | C | A | T | G | 0 | 0 | 2 | AJ490310 |

[a]This SNP determines whether the protein has 23-Ala (G) or 23-Thr (A)
[b]This SNP determines whether the protein has 164-Ile (A) or 164-Val (G)
[c]For an additional two Museum samples the amplification was only successful for part 3 of the gene (698-1117 bp). Based on the SNPs found, these may represent either allele Crcr-CytB*1 or Crcr-CytB*2

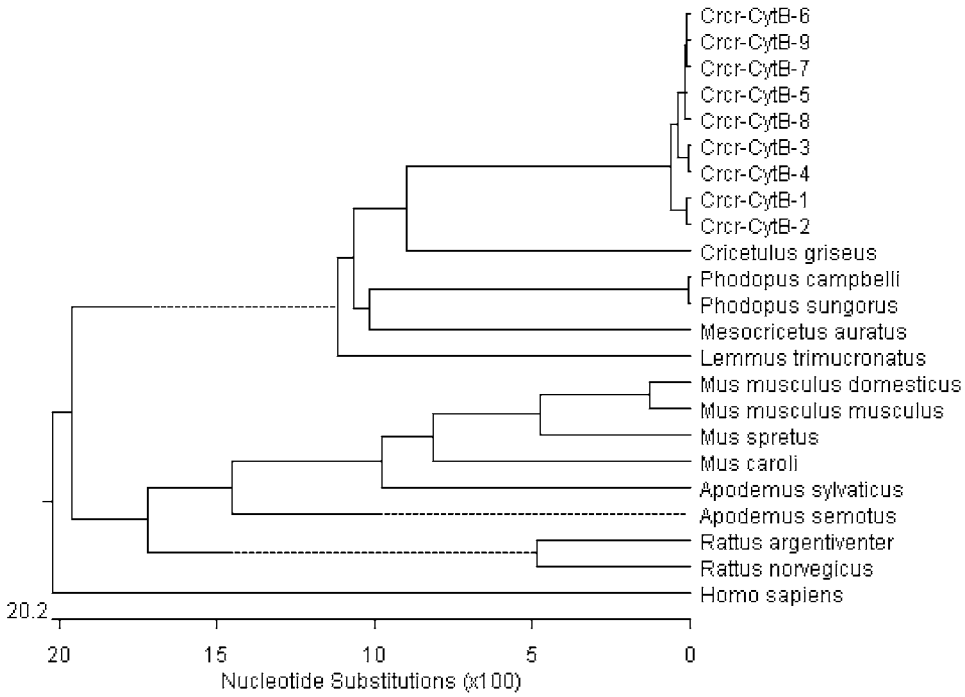


*Figure 1.* UPGMA tree of cytochrome B sequences, including the nine Cricetus cricetus sequences of this study (accession numbers in Table 1) and some other Rodent species, with one human sequence as outgroup. All available full-length sequences from the Cricetinae clade have been included (AB033693 Cricetulus griseus, AF119265 *Mesocricetus auratus*, AF119266 *Phodopus sungorus*, and AF119278 *P. campbelli*). *Lemmus trimucronatus* (AF119276) belongs to the Arvicolinae clade of the Muridae family. Other accession numbers: *M. musculus musculus* (AB033699), *M. musculus domesticus* (AB042432), *M. spretus* (AB033700), *M. caroli* (AB033698), *A. sylvaticus* (AB033695), *A. semotus* (AB033694), *R. norvegicus* (AB033702), *R. argentiventer* (AB033701), *H. sapiens* (V00662). Alignment with Clustal V.

but we managed to obtain the complete sequence of Crcr-CytB*1 in three samples, and partial sequences in two samples. The partial sequences could be either Crcr-CytB*1 or Crcr-CytB*2. The number of animals for which sequence information is available is too small to be certain about the allelic diversity in the historic Dutch population, although the results are consistent with the presence of allele Crcr-CytB*1 only. In contrast, nine different alleles were present in the 15 Czech samples. Unfortunately, amplification of this gene was not successful in the French samples.

Only two of the 20 SNPs translated into amino acid variants. One mutation, 23-Ala → 23-Thr, was also present in the Muridae species *Mesocricetus auratus* (AF119265), *Peromyscus maniculatus* (AF119261) and *Tokudaia osimensis* (AB033703). The other one was the replacement of isoleucine (164) by valine. Although it does not seem likely that this would affect the function of the protein, the replacement does occur in a highly conserved region, which was exactly identical in all closely related Rodent species. When a part of this conserved amino acid sequence (PYIGTTLVEWVWGGFSVDKATL, valine in question is underlined) was blasted against the EMBL database, only 15 out of around 1000 entries had a valine in the same position. Well over 950 sequences had an isoleucine at this position. The valine was found in a variety of species, including dolphins (*Lipotes vexillifer, Inia geoffrensis*), whales (*Eschrichtius robustus, Kogia simus, Kogia breviceps*), and one primate (*Nycticebus coucang*). It was present in *Meles meles* (Eurasian badger), a species with known low genetic diversity, and in only one of 30 different cytochrome B sequences in *Microtus lingicaudus* (long-tailed vole). Also in C. cricetus the 164-Ile → 164-Val was present in only one (Crcr-CytB*1) of 9 different alleles. This allele was found only once in the 14 samples of the Czech population. It was present in the Museum animals, and has become fixed in the Dutch current population.

*MHC diversity*

The DQA sequences of the Common Hamster were 66.9–79.7% (exon 2) and 84.9–90.0% (exon 3) identical at the DNA level to the human, rat and mouse sequences, and somewhat less at the amino acid level, indicating that the amplified sequences most likely represent the functional equivalent of this gene. In contrast to other rodent species (Pfau et al. 2001), no variation was found among the *C. cricetus* samples in DQA exon 2 and 3 (sequences deposited in the EMBL genebank under accession numbers AJ490325 and AJ490326). For DRB exon 2, one band was amplified, and this could be sequenced directly in all animals, producing a readable sequence. If some nucleotides were unclear, cloning of the PCR product of that animal and sequencing of up to 10 clones always produced two different, related sequences varying at the expected positions. We never detected more than two different sequences in one animal, and in our analysis we assume that the alleles are from one locus. All but one of the alleles found in museum samples were also found in samples from living animals, which contained much more DNA. The one exception was an allele (Crcr-DRB*14) closely related to others found here. Therefore, it is unlikely that museum samples were contaminated during extraction.

In DRB exon 2, we observed a total of 39 SNPs in 240 bp, forming 14 different alleles (Table 2). This corresponded to a total of 19 amino acid differences, evenly distributed between synonymous (9) and non-synonymous (10) mutations. From Table 3, it appears that alleles have exchanged SNPs, possibly through crossing-over events. The Common Hamster alleles cluster in a group, loosely with mouse and rat DRB genes, but separate from a series of non-Rodent outgroup sequences (Figure 2). This type of clustering is not uncommon for DRB genes, it is comparable to, e.g., sheep and goat DRB exon 2 sequences (Jugo and Vicario 2000).

The Dutch museum samples and the Czech population contained a large number of alleles (Figure 2), consistent with the large genetic diversity at MHC loci found in a range of species. In the 20 museum samples, 8 different alleles were found. Eleven animals were heterozygous for this locus (Table 3). The sample of 15 animals from the current Czech population contained 13 different alleles, twelve animals were heterozygous. Under the assumption that the Dutch museum animals represent a sample from one historic population, we can compare Dutch and Czech diversity in a population of considerable size. Then, the difference in number of alleles between the current Czech and historical Dutch groups is not significant (Fisher's exact test, $P = 0.26$), and the level of heterozygosity is comparable. $F_{st}$ was 0.079. All but one allele present in the Dutch museum animals were also found in the current Czech samples. It is not unlikely that the allele missing is present in the Czech population but was not included in our sample as the

*Table 2.* SNPs found in DRB exon2

| | Nucleotide position | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Number of times found[a] | EMBL accession number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 38 | 39 | 41 | 44 | 45 | 50 | 51 | 52 | 53 | 62 | 63 | 65 | 71 | 75 | 88 | 102 | 125 | 127 | 130 | 131 | 132 | 133 | 143 | 144 | 145 | 151 | 160 | 161 | 164 | 174 | 175 | 181 | 194 | 195 | 218 | 219 | 220 | 223 | | |
| Crcr-DRB*01 | T | A | T | G | A | T | A | C | A | C | A | G | A | T | T | T | C | G | G | G | A | C | T | T | C | T | C | T | A | A | G | G | G | T | C | T | T | C | 27[b] | AJ490311 |
| Crcr-DRB*02 | C | T | G | G | T | G | T | A | G | C | A | G | A | C | C | T | C | G | G | G | A | C | T | G | G | C | C | T | A | A | G | G | G | T | C | T | T | C | 3 | AJ490312 |
| Crcr-DRB*03 | C | T | G | G | T | T | A | C | A | C | A | G | A | C | C | T | C | G | G | G | A | C | T | G | G | C | G | C | C | A | G | G | G | T | C | T | T | C | 10 | AJ490313 |
| Crcr-DRB*04 | G | G | G | G | T | T | A | C | A | C | A | G | A | T | C | T | C | G | G | G | A | C | T | G | G | C | G | C | C | A | G | G | G | T | C | T | T | C | 12 | AJ490314 |
| Crcr-DRB*05 | C | T | G | G | A | T | A | C | T | C | G | G | T | C | C | T | C | G | G | G | A | C | T | G | G | C | G | C | C | A | G | G | G | T | C | T | T | C | 3 | AJ490315 |
| Crcr-DRB*06 | C | T | G | G | A | T | A | C | T | C | G | G | T | T | T | T | C | G | G | G | A | C | T | G | G | C | G | C | C | A | G | G | G | T | C | T | T | C | 2 | AJ490316 |
| Crcr-DRB*07 | T | T | C | C | A | T | A | C | T | G | G | A | T | T | C | A | C | G | C | T | C | A | T | G | G | C | G | C | C | G | A | C | T | A | G | G | G | G | 4 | AJ490317 |
| Crcr-DRB*08 | T | T | C | C | A | T | A | C | T | G | G | A | T | T | C | A | A | A | C | T | C | A | T | G | G | C | G | C | C | G | A | C | T | A | G | G | G | G | 4 | AJ490318 |
| Crcr-DRB*09 | T | T | C | C | A | T | A | C | T | G | G | A | T | T | C | A | A | A | C | T | C | A | T | T | C | T | G | C | C | G | A | C | T | A | G | G | A | G | 2 | AJ490319 |
| Crcr-DRB*10 | C | T | G | G | T | G | T | A | G | C | A | G | T | T | T | T | A | A | C | T | C | A | T | T | C | T | G | C | C | A | G | G | T | A | G | G | A | G | 2 | AJ490320 |
| Crcr-DRB*11 | C | T | G | G | T | T | A | C | T | C | G | G | T | C | C | T | C | G | C | T | C | A | T | G | G | C | G | C | C | A | G | G | G | T | G | G | A | G | 1 | AJ490321 |
| Crcr-DRB*12 | T | A | G | G | T | T | A | C | A | C | A | G | A | T | C | T | C | G | G | G | A | C | C | T | C | T | C | A | A | A | G | G | G | T | G | G | A | G | 4 | AJ490322 |
| Crcr-DRB*13 | C | T | G | G | T | T | A | C | T | C | G | G | T | C | C | T | C | G | G | G | A | C | T | G | G | C | C | T | A | A | G | G | G | T | G | G | A | G | 1 | AJ490323 |
| Crcr-DRB*14 | G | G | G | G | T | T | A | C | G | C | A | G | A | C | C | T | C | G | G | G | A | C | T | T | C | T | C | T | A | A | G | G | T | A | G | G | A | G | 1 | AJ490324 |

[a]See Figure 2 for the distribution of alleles across groups of samples
[b]of which 13 times in current Dutch animals.

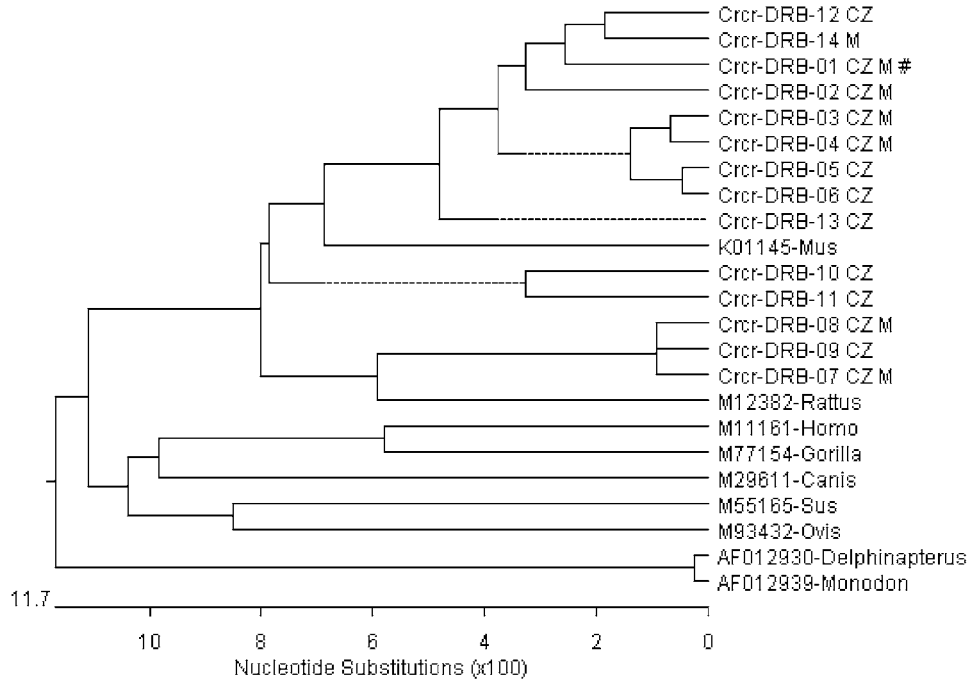


*Figure 2.* UPGMA tree (Clustal V) of DRB alleles of *C. cricetus* and a number of DRB exon 2 sequences from other species. For the Common Hamster alleles, next to the allele designation, the occurrence in three groups of samples is indicated: CZ: Czech population; M: Dutch museum samples; #: allele in the recent Dutch population.

number of samples taken was small in comparison to the large number of alleles found.

In the recent Dutch and French Hamster populations, all 16 animals were identical, having a single allele, designated Crcr-DRB*01. All animals were homozygous. The allele Crcr-DRB*01, fixed in the current Dutch and French animals, was also present in the Czech population, but at a frequency of only 0.1 (Table 3). In the museum samples, the allele was present in samples from each period of time. This is in line with the average frequency of 0.33, and it indicates that the allele was always relatively abundant. Nevertheless, these museum animals contained 7 other alleles, all of which have been lost in the current population.

## Discussion

The 13 current Dutch Common Hamster animals included in this study are part of a breeding program. It is debated whether any free-living animals remain in the field in the South of the Netherlands, although they do occur in neighbouring areas in Belgium and Germany. These 13 animals were compared to samples from stuffed animals in museum collections collected in different periods during the last century, taken as a representative sample of the diversity in the historic population. A few samples were obtained from the current French population in Alsace, which would belong to the same putative subspecies. We experienced problems in obtaining samples due to the fact that this species is lawfully protected, and capture is prohibited. As a result, no samples from Belgium or the Western part of Germany could be obtained. As a reference for the degree of polymorphism to be expected in a large population, and as a reference for the major putative subspecies, we used samples from animals in a densely populated area around Brno in Czech Republic.

We posed two questions: can we find evidence for a subspecies status of the Dutch animals, and did they experience a loss of genetic diversity?

### *Is there evidence for a separate subspecies?*

As stated in the introduction, the idea of a separate subspecies status for the Hamsters in Western Europe goes back to 1899, but solid evidence has been difficult to obtain (Husson 1959; Grulich 1987). We employed

*Table 3.* Sample size, number of alleles, observed ($H_o$) and expected ($H_e$) heterozygosity for DRB exon 2 in the groups of samples used in this study. $H_e$ was calculated assuming that the samples were a random sample from one population

| Population | Sample size (animals) | Number of alleles found | Number of genotypes found | $H_o$ | $H_e$ | Frequency of allele Crcr-DRB*01 |
|---|---|---|---|---|---|---|
| Recent Czech population near Brno | 15 | 13 | 12 | 0.80 | 0.88 | 0.10 |
| Recent French population in Alsace | 3 | 1 | 1 | 0.00 | 0.00 | 1.00 |
| Recent Dutch animals (1980–2000) | 13 | 1 | 1 | 0.00 | 0.00 | 1.00 |
| Dutch museum specimens, including | 20 | 8 | 11 | 0.60 | 0.75 | 0.32 |
| *Animals caught near Houthem (1924–1928)* | *12* | *6* | *7* | *0.67* | *0.77* | *0.29* |
| *Animals caught near Oud-Valkenburg (1938)* | *4* | *5* | *4* | *0.75* | *0.79* | *0.13* |
| *Animal caught near Heerderberg (1947)* | *1* | *1* | *1* | *0.00* | *0.00* | *1.00* |
| *Animals caught near Heer (1954–1956)* | *3* | *2* | *3* | *0.33* | *0.60* | *0.50* |

molecular methods studying cytochrome b diversity of the mitochondrial genome. The data obtained here using museum animals clearly show that the Dutch population in the first half of the 20th century shared all alleles with the Czech population, Therefore, the data give no support for two monophyletic evolutionary significant units (ESUs). This is in agreement with Niethammer (1982) and Grulich (1987), who consider the animals in Belgium and The Netherlands as the end of a cline in body size and some other characteristics, at the edge of the range of the species *C. cricetus*.

When compared to the average nucleotide differences among rodent species, the different Hamster cytochrome b alleles can be considered to be highly similar. Conroy and Cook (1999) found 45 variable amino acids in the cytochrome b of 111 specimens of *Microtus longicaudus*, while we found only 2 variable amino acids in 51 animals. As can be seen in Figure 1, the differences among the Hamster alleles are also much smaller than the differences found between, e.g., two *Mus musculus* subspecies.

*Loss of functional diversity*

Most natural mammal populations that have been studied possess abundant MHC diversity, both in terms of the number of alleles present and the extent of sequence variation among alleles (Klein 1986; Potts and Wakeland 1990; Nei and Hughes 1991; Hedrick 1994). The diversity in the DRB gene exon 2 among the Czech animals is consistent with this notion: in 15 animals we found 13 different alleles. Almost all animals had a unique genotype, almost all were heterozygous at this locus. The variation in the historical Dutch samples was somewhat less, but still extensive (8 different alleles, 60% of the animals heterozygous at this locus). In contrast, the variation in the current Dutch animals was completely lost: only one allele was present, and as a consequence all animals were homozygous at this locus and identical to each other. This indicates that the current Dutch animals have become fixed for one DRB allele.

Although diversity at MHC loci is believed to have adaptive significance, very low MHC diversity does occur in some taxa such as moose (Mikko and Andersson 1995; Mikko et al. 1999). Therefore, at least at the short term, low MHC diversity does not necessarily lead to increased susceptibility to disease. Hughes (1991) argued that the main goal of a captive breeding program should be to maintain the allelic diversity at MHC loci. However, his view was challenged by others, fearing loss of genetic diversity at many other, equally important loci if the program would be targeted at maximising allele diversity at MHC loci (Vrijenhoek and Leberg 1991; Gilpin and Wills 1991; Miller and Hedrick 1991). For the Dutch Common Hamsters studied here, the loss of genetic diversity at an MHC locus that until recently maintained excessive polymorphism, as well as the loss of diversity at microsatellite loci (Neumann et al., in prep.), suggests that a severe bottleneck has taken place. The lack of diversity could be genome-wide, with possibly fixation of deleterious recessive mutations at some loci. Backbier et al. (1998) report of studies indicating reduced vitality and fertility in Dutch and German Common Hamster populations. This would be a strong justification for enhancing

genetic diversity by introducing new individuals into the population.

*Consequences for a breeding program*

From a conservation point of view, one should not mix subspecies in breeding programs unless absolutely necessary. Since subspecies often occur locally or regionally and, hence, are usually in lower numbers than the species as a whole, it follows that one should protect subspecies even more. Indeed, such was noted in the protection plan for the Common Hamster in the Netherlands (Krekels 1999). We obtained no molecular evidence in the cytochrome b diversity that would support this taxonomic status. The set of mitochondrial haplotypes found in the present-day Czech population overlapped nearly completely with that found in the Dutch samples that are more than 40 years old. For the MHC alleles, the Dutch museum samples contained a fair amount of diversity (8 alleles in 20 animals), and these alleles were a subset of the allelic diversity in the (much larger) Czech population. Unfortunately, since trans-species polymorphisms are known to occur at these loci, the MHC data are uninformative regarding the subspecies question.

In conclusion, we found no evidence to support a separate conservation status for western European Hamsters. We did find complete loss of genetic diversity at a MHC locus that until recently maintained excessive polymorphism. In combination with the loss of diversity at microsatellite loci (Neumann et al., in prep.), this suggests that the loss of diversity due to a bottleneck could be genome-wide. The likely fixation of deleterious recessive mutations at other loci would be a strong justification for introducing new individuals into the ongoing breeding program. Introducing central and eastern European animals in the breeding program would be one way of doing this.

Alternatively, the remaining animals, and those raised in the captive breeding program, may mix and breed in the field with animals dispersing from populations in Germany and Belgium. However, in these areas the number of animals has also dwindled (Backbier et al. 1998), and it is not clear how many animals remain. In addition, it is not known whether they experienced a similarly severe bottleneck. This information needs to be obtained, since it is crucial for management decisions to obtain a sustainable population. In any event, an increase of MHC diversity in the Dutch population will be easily detectable against the current monomorphic background. It is therefore feasible to include it in the monitoring program that will be established to examine the effects of reintroduction in the field.

## Acknowledgements

H. Jansman, R. van Apeldoorn, K. Neumann and I. Losinger are thanked for their generous gift of DNA samples, and Johan Thissen for suggestions and critical reading of the manuscript. This research was supported by the Netherlands' Ministry of Agriculture, Nature Management and Fisheries.

## References

Backbier LAM, Gubbels EJ, Seluga K, Weidling A, Weinhold U, Zimmermann W (1998) *Der Feldhamster Cricetus cricetus (L. 1758). Eine stark gefährdete Tierart*. Internationale Arbeitsgruppe Feldhamster. Stichting Hamsterwerkgroep Limburg, Margraten, The Netherlands. 32 pp. ISBN 90-76465-01-0.

Booy G, Hendriks RJJ, Smulders MJM, Van Groenendael JM, Vosman B (2000) Genetic diversity and the survival of populations. *Plant Biol*., **2**, 379–395.

Brown JL, Eklund A (1994) Kin recognition and the major histocompatibility complex: An integrative review. *American Naturalist*, **143**, 435–461.

Conroy CJ, Cook JA (1999) Mt DNA evidence for repeated pulses of speciation within arvicoline and murid rodents. *J. Mamm. Evol*., **6**, 221–245.

Conroy CJ, Cook JA (2000) Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Rodetia: Muridae). *Mol. Ecol*., **9**, 165–175.

Ditchfield AD (2000) The comparative phylogeography of Neotropical mammals: Patterns of intraspecific mitochondrial DNA variation among bats contrasted to nonvolant small mammals. *Mol. Ecol*., **9**, 1307–1318.

Dupond Ch (1932) La propagation du hamster en Belgique. *Bulletin du Musée Royal d'Histoire naturelle de Belgique/Mededeelingen van het Koninklijk Natuurhistorisch Museum van België*, **8**(26), 1–43.

Edwards ST, Potts WK (1996) Polymorphism of genes in the major histocompatibility complex (MHC): Implications for conservation genetics of vertebrates. In: *Molecular Genetic Approaches in Conservation* (eds. Smith TB, Wayne RK), pp. 214–237. Oxford University Press, New York.

Ellegren H, Mikko S, Wallin K, Anderson L (1996) Limited polymorphism at major histocompatibility complex loci in the Swedish moose A. alces. *Mol. Ecol*., **5**, 3–9.

Freeland JR, Noble LR, Okamura B (2000) Genetic diversity of North American populations of *Cristatella mucedo*, inferred from microsatellite and mitochondrial DNA. *Mol. Ecol*., **9**, 1375–1389.

Gilpin M, Wills C (1991) MHC and captive breeding: A rebuttal. *Cons. Biol*., **5**, 554–555.

Grulich I (1987) Variability of *Cricetus cricetus* in Europe. *Acta Sc. Nat. Brno*, **21**, 1–53.

Haig SM (1998) Molecular contributions to conservation. *Ecology*, **79**, 413–425.

Hedrick PW (1994) Evolutionary genetics of the major histocompatibility complex. *American Naturalist*, **143**, 945–964.

Higgins DG, Sharp PM (1989) Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS*, **5**, 151–153.

Hill AVS, Allsopp CEM, Kwiatowski D (1991) Common West African HLA antigens associated with protection from severe malaria. *Nature*, **356**, 595–600.

Hughes AL (1991) MHC polymorphism and the design of captive breeding programs. *Cons. Biol*., **5**, 249–251.

Husson AM (1959) On the systematic position of the western Hamster, Cricetus cricetus canescens Nehring (Mammalia: Rodentia). *Bijdragen tot de dierkunde*, **29**, 187–201.

Jugo BM, Vicario A (2000) Single-strand conformational polymorphism and sequence polymorphism of Mhc-DRB in Latxa and Karrantzar sheep: Implications for Caprinae phylogeny. *Immunogenetics*, **51,** 887–897.

Kayser A, Stubbe M (2000) Colour variation in the common Hamster Cricetus cricetus in the north-eastern foot-hills of the Harz Mountains. *Acta Theriologica*, **45**, 377–383.

Klein J (1986) *Natural History of the Major Histocompatibility Complex*. Wiley & Sons, New York.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Evolution*, **86**, 6196–6200.

Krekels, R (1999) *Beschermingsplan Hamster 2000–2004*. Rapport Directie Natuurbeheer nr. 41. Wageningen, The Netherlands, Minstry of Agruculture, Nature conservation and Fisheries, IKC Natuurbeheer, 60 pp (in Dutch).

Malchoff DM, Brufsky A, Reardon G, McDermott P, Javier EC, Bergh C-H, Rowe D, Malchoff CD (1993) A mutation of the glucocorticoid receptor in primary cortisol resistance. *J. Clin. Invest*., **91**,1918–1925.

McGuire KL, Duncan WR, Tucker PW (1985) Syrian hamster DNA shows limited polymorphism at class 1-like loci. *Immunogenetics*, **22**, 257–268.

Mikko S, Andersson L (1995) Low major histocompatibility comples class II diversity in European and North American moose. *Proc. Natl. Acad. Sci. USA*, **92**, 4259–4263.

Mikko S, Roed K, Schmutz S, Andersson L (1999) Monomorphism and polymorphism at Mhc DRB loci in domestic and wild ruminants. *Immunol. Rev*., **167**, 169–178.

Miller PB (1998) *TFPGA: Tools for Population Genetic Analyses for Windows*. Arizona State University.

Miller PS, Hedrick PW (1991) MHC polymorphism and the design of captive breeding programs: Simple solutions are not the answer. *Cons. Biol*., **5**, 556–559.

Nei M, Hughes AL (1991) Polymorphism and evolution of the major histocompatibility complex loci in mammals. In: *Evolution at the Molecular Level*, pp. 222–247. Sinauer Associates, Sunderland.

Niethammer J (1982) *Cricetus cricetus* (Linneaus, 1758) – Hamster (Feldhamster). In: *Handbuch des Sáugetiere Europas, Band 2/I, Rodentia II* (eds. Niethammer J, Krapp F), pp. 7–28. Akademische Verlagsgesellschaft, Wiesbaden, Germany.

Passbøl PJ, Arctander P (1998) Primers for animal mitochondrial DNA: The importance of species-specific primers. In: *Molecular Tools for Screening Biodiversity* (eds. Karp A, Isaac PG, Ingram DS), pp. 249–255. Chapman & Hall, London.

Pfau RS, Van den Bussche RA, McBee K (2001) Population genetics of the cotton rat (*Sigmodon hispidus*): Patterns of genetic diversity at the major histocompatibility complex. *Mol. Ecol*., **10**, 1939–1945.

Potts W, Penn D (1998) MHC-disassortive mating preferences reversed by cross-fostering. *Proc. Royal Soc. London B*, **265**, 1299–1306.

Potts WK, Wakeland EK (1990) Evolution of diversity of the major histocompatibility complex. *Trends Ecol. Evol*., **5**, 181–187.

Potts WK, Wakeland EK (1993) Evolution of MHC genetic diversity: A tale of incest, pestilence and sexual preference. *Trends Genet*., **9**, 408–412.

Ryder OA 1986 Species conservation and systematics: The dilemma of subspecies. *Trends Ecol. Evol*., **1**, 9–10

Sanjayan MA, Crooks K, Zegers G, Foran D (1996) Genetic variation and the immune response in natural populations of pocket gophers. *Cons. Biol*., **10**, 1519–1527.

Stubbe M, Stubbe A (1998) *Ecology and Protection of the Common Hamster*. Martin-Luther-Universität, Halle-Wittemberg, Halle/ Saale.

Suzuki H, Tsuchiya K, Takezaki N (2000) A molecular Phylogenetic Framework for the Ryukyu Endemic Rodents *Tokudaia osimensis* and *Diplothrix legata*. *Mol. Phylogenet. Evol*., **15**, 15–24.

Vrijenhoek RC, Leberg PL (1991) Let's not throw the baby out with the bath water: A comment on management for MHC diversity in captive populations. *Cons. Biol*., **5**, 252–254.

Wallis GP (1994) Population genetics and conservation in New Zealand: A hierarchical synthesis and recommendations for the 1990s. *J. Royal Soc. New Zealand*, **42**, 143–160.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506–513.

Wayne RK, Jenks SM (1991) Mitochondrial DNA analysis implying extensive hybridization of the endangerd red wolf *Canis rufus*. *Nature*, **351**, 565–568.

Wayne RK, Meyer A, Lehman N, Van Valkenburgh B, Kat PW, Fuller TK, Girman D, O'Brien SJ (1990) Large sequence divergence among mitochondrial DNA genotypes within populations of eastern African black-backed jackals. *Pop. Biol*., **87**, 1772–1776.

Wenink PW, Groen AF, Roelkepakker ME, Prins HHT (1998) African buffalo maintain high genetic diversity in the major histocompatibility complex in spite of historically known population bottelenecks. *Mol. Ecol*., **7**, 1315–1322.

Wilbur J, Lipman D (1984) The context dependent comparison of biological sequences. *SIAM J. Apppl. Math*., **44**, 557–567.

Zhang Y (1997) *Distribution of Mammalian Species in China*. China Forestry Publishing House, Beijing.