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Genetic spatial structure of European common hamsters (*Cricetus cricetus*) — a result of repeated range expansion and demographic bottlenecks

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Abstract

The spatial genetic structure of common hamsters (*Cricetus cricetus*) was investigated using three partial mitochondrial (mt) genes and 11 nuclear microsatellite loci. All marker systems revealed significant population differentiation across Europe. Hamsters in central and western Europe belong largely to two allopatric mitochondrial lineages south and northwest of the Carpathian and Sudetes. The southern group, 'Pannonia', comprises populations inside the Carpathian basin (Czech Republic, Hungary) while the second group, 'North', includes hamsters from Belgium, the Netherlands, France, and Germany. Isolation of the lineages is maintained by a combination of geographical and ecological barriers. Both main phylogeographical groups show signs of further subdivision. North is separated into highly polymorphic central German and less polymorphic western populations, which most likely split during late glacial expansion (15 000–10 000 BP). Clock estimates based on haplotype distributions predict a divergence of the two major lineages 85 000–147 000 BP. Expansion times fall during the last glaciation (115 000–10 000 BP) corroborating fossil data, which identify *Cricetus cricetus* as characteristic of colder climatic phases. Despite the allopatry of mt haplotypes, there is an overlap of nuclear microsatellite alleles between phylogeographical units. Although there are strong evidence that Pannonian hamsters have persisted inside the Carpathian basin over the last 50 000 years, genetic differentiation among European hamsters has mainly been caused by immigration from different eastern refugia. Possible source populations are likely to be found in the Ukrainian and the southern Russian plains — core areas of hamster distribution. From there, hamsters have repeatedly expanded during the Quaternary.

Keywords: bottlenecks, common hamster, glacial refugia, phylogeography, postglacial expansion

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Introduction

The oscillation of warm and cold phases during the Quaternary has promoted extensive shifts of distribution areas, and hence population diversity in many temperate animals (Cooper *et al*. 1995; Taberlet *et al*. 1998; Seddon *et al*. 2001). Survival in separate refugia has led to the allopatric formation of differing genetic lineages during stadials. Glacial refugia for European small mammals were mainly located in the Mediterranean, the Balkans, the Urals and the Caucasus/Carpathian region (Markova *et al*. 1995; Hewitt 1996; Taberlet *et al*. 1998; Jaarola & Searle 2002) with additional retreat areas in central Europe (Bilton *et al*. 1998; Brunhoff *et al*. 2003). Range expansion caused further genetic differentiation (Conroy & Cook 2000a; Milá *et al*. 2000) because of serial bottlenecking in founder populations and the selection of alleles favourable in a novel environment (Rogers 1995; Ibrahim *et al*. 1996; Hewitt 1999). Species arriving in new habitats were still confronted with

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climatic changes. This happened, in particular, towards the end of the last glacial (16 000 BP) and the beginning of the Holocene (10 000 BP) as this period was characterized by several warm and cold (Dryas) spells. Spreading woods and increased humidity during the Atlantikum (7500–4500 BP) and early Sub-Atlantikum (2800–2000 BP) must have caused habitat losses for taxa adapted to arid continental climates

Phylogeographical studies on small rodents have focused mainly on species with relatively wide and northerly reaching distributions (Jaarola & Tegelström 1995; Jaarola & Searle 2002; Brunhoff *et al*. 2003). Despite similarities, such as the importance of southern European refugia, there is a large species-specific variance among phylogeographical patterns (Haynes *et al*. 2003; Michaux *et al*. 2003).

To complement these studies on small rodents, we here aim to infer the genetic phylogeography of the common hamster, *Cricetus cricetus*, which is somewhat different from most of the previously analysed muroids in its ecological preferences and adaptability. The main distribution area of this semifossorial and facultatively hibernating animal lies in the eastern European and western Asian plains where it occupies steppe, meadows and steppe-forests (Berdyugin & Bolshakov 1998; Nechay 2000). The northern species boundary roughly coincides with 55° northern latitude, although in Russia it extends up to 59° northern latitude (Niethammer & Krapp 1982). Common hamsters in western and central Europe are largely restricted to agricultural sites with deep loess soils and suitable microclimates (Grulich 1975; Nechay *et al*. 1977). Palaeontological data suggest that *C. cricetus* underwent repeated range shifts during the Quaternary (Storch 1974; Markova *et al*. 1995; Kowalski 2001). Wood clearances during Neolithic and medieval times created the last significant advances of the common hamster (Dupont 1932; Werth 1936; Clason 1999). Formerly highly abundant, hamsters have suffered from a Europe-wide population reduction over the last 40 years (Backbier & Gubbels 1998; Murariu 1998; Nechay 2000). The most dramatic population collapses have occurred along the western frontier of the distribution (Libois & Rosoux 1982; Baumgart 1996). A previous study has shown that genetic depauperization of western hamsters, which are considered to represent the subspecies *Cricetus cricetus canescens* (Mitchell-Jones *et al*. 1999), is not only caused by the current decline but also historical events (Neumann *et al*. 2004). Changes in agricultural management are suspected to provide the main reason for the progressive disappearance of the species (Nechay *et al*. 1977; Nechay 2000) but potential climatic effects have not yet been examined.

We conducted this study to investigate the following questions: Does the phylogeographical structure of the common hamster match that of other European muroids? Is it in agreement with a late/postglacial expansion model and, if so, where are the locations of potential refugia? Did population structure evolve under ecological and geographical constraints?

Exploring the glacial history of common hamsters not only supports species-specific conservation measures but may also enhance our general understanding of population developments along species boundaries.

Materials and methods

Population sampling

Sampling concentrated mainly on populations in central and western Europe covering the range of the two subspecies, *Cricetus cricetus canescens* and *Cricetus cricetus cricetus*. Additionally, we included hamsters from European Russia, as well as single specimens each from Romania, Poland and western Siberia. A total of 435 specimens were collected from more than 60 localities in eight countries. Table 1 shows sampling localities and the number of individuals examined. Figure 1 gives details of geographical distributions.

DNA extraction

Genomic DNA isolation from fresh or ethanol fixed materials such as ear, liver, muscle, hair and skin followed a standard protocol supplied with the E.Z.N.A. Tissue DNA Kit II system (Peqlab Biotechnologie).

Mitochondrial DNA analysis

We investigated three partial mitochondrial (mt) regions: control region (*ctr*), 16S rRNA (*16S*) and cytochrome *b* (cyt *b*). Before the experiment, we compared polymerase chain reaction (PCR) products obtained from hair, ear and liver of the same animal to exclude tissue-specific amplification of pseudogenes. No additional gene copies were found. All three amplified mt genes proved either similar or almost identical to previously published Cricetinae sequences (Nakamichi *et al*. 1998; Hashiguchi & Ikushima 1998; Smulders *et al*. 2003). To avoid extensive sequencing, we did not use the same number of individuals for every gene. Control region sequences obtained in a previous study were included (Neumann *et al*. 2004). PCR amplification, purification and sequencing of DNA followed largely the procedure described in Neumann *et al*. (2004). A PCR product of 421 bp was amplified.

Two novel internal sequencing primers were designed for the control region (DlInt1: 5′-ATTCCCCTAGCATATAAGCAT-3′, annealing temperature 50 °C; DlInt2: 5′-GTGGGCGGGTTGCTGGTTTCT-3′, annealing temperature 60 °C).

Partial 16S rRNA (554 bp total fragment length) was amplified and sequenced using original shrew primers at 54 °C annealing temperature (Quérouil *et al*. 2001).

Table 1 Sampling locations of European common hamsters and numbers of individuals included in mitochondrial (*ctr*) and microsatellite analyses. Note that not all animals were investigated for all three mt genes

			Analysed individuals		
Sampling region	Location (country)	Sample ID	Total	mt Loci *ctr*	Microsatellites
Western populations	Limburg (the Netherlands)	W1	28	18	28
	Flanders (Belgium)	W2	10	9	10
	Alsace (France)	W3	67	20	67
	Northrhine-Westfalia (Germany)	W4	7	3	7
	Baden-Wuerttemberg (Germany)	W5	33	20	32
	Rhineland-Pfalz (Germany)	W6	2	2	2
	Hessen (Germany)	W7	1	1	—
Central German populations	Lower Saxony (Germany)	C1	24	18	17
	Saxony-Anhalt (Germany)	C2	97	20	97
	Thuringia (Germany)	C3	35	20	35
Carpathian Basin/Pannonia	Southern Moravia (Czech Republic)	P1	65	24	65
	seven locations across Hungary	P2	40	25	40
Other samples from Europe/Asia	Brzezie (Poland)	E1	1	1	1
	Craiova (Romania)	E2	1	1	—
	Mozdok/Caucasus (Russia)	E3	2	2	1
	Saratov (Russia)	E4	1	1	—
	Kirov (Russia)	E5	2	2	2
	Ural/Ekaterinburg (Russia)	E6	19	12	19
	Novosibirsk (Russia)	E7	1	1	1
Total			435	200	424

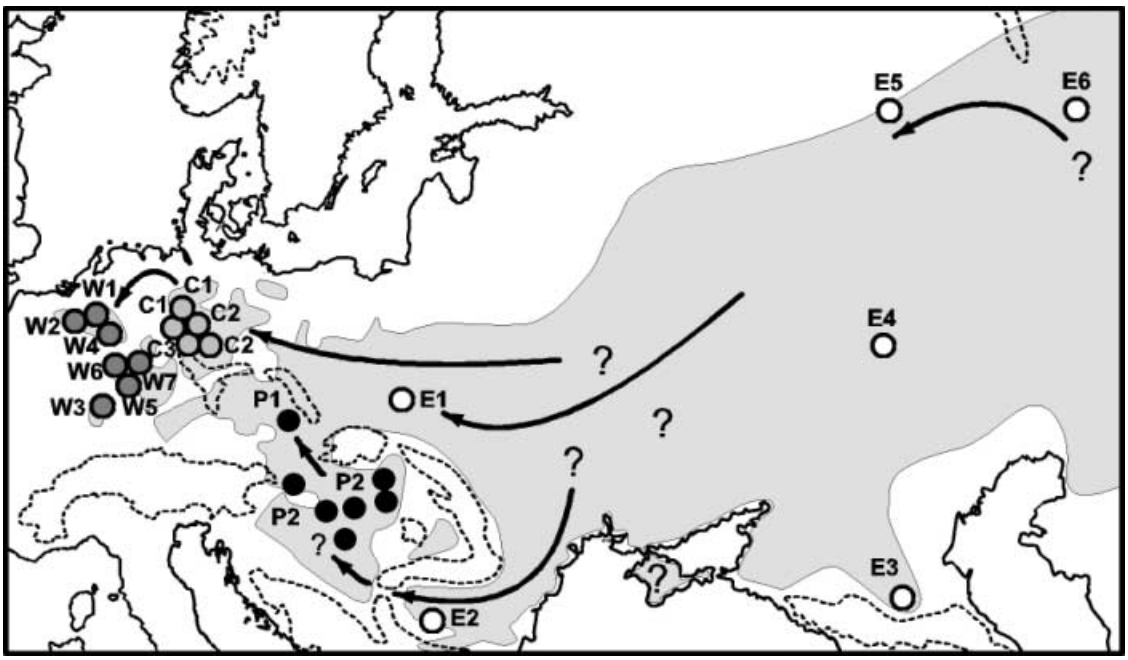

Fig. 1 Geographical distribution of *Cricetus cricetus* samples (circles) and proposed expansion routes (arrows). Grey areas refer to the recent distribution range according to Panteleyev (1998) and Mitchell-Jones *et al*. (1999). Question marks indicate potential glacial refugia deduced from fossil records (Markova *et al*. 1995). Legend: circles (dark grey), West; light grey, Central; black, Pannonia; white, Poland, Romania, Russia. See Table 1 for sample symbols.

Cytochrome *b* (984 bp total fragment length) was amplified using primers L14841 (Kocher *et al*. 1989) and HCRIC3 (5′-GATGAAAGGGTATTCTACTGGTTG-3′) at 50 °C annealing temperature. Sequencing was carried out using flanking and internal primers (CbLint1: 5′-ACGTACTAC-CATGAGGTCAAAT-3′, annealing temperature 51 °C; CbLint2: 5′-TCCCGCACACATTAAACC-3′, annealing temperature 50 °C; CbHint1: 5′-GTGGATTTGCAGGAG-TATAAT-3′, annealing temperature 50 °C; CbHint2: 5′-AATGATTTGGCCTCATGGGAG-3′, annealing temperature 53 °C; CbHint3: 5′-CGGCAGATGTGGGTTACTGAT-3′, annealing temperature 58 °C).

Sequences were aligned in PROSEQ (version 2.9, D.A. Filatov, University of Birmingham, UK) and the number of haplotypes (N_H) scored. Nucleotide diversity π (based on haplotypes in percentage) within groups and net distance D_a (%) between phylogroups were calculated using the Kimura 2-parameter (K2P) method (MEGA 2.1, Kumar *et al*. 2001). Net distance corrects distance measures between groups by subtracting mean within-group distances. Parsimony (Templeton *et al*. 1992) and median-joining (Bandelt *et al*. 1999) networks were constructed using TCS 1.18 software (Clement *et al*. 2000) and NETWORK 4101 (www.fluxus-engineering.com), respectively. Minimum evolution (ME: K2P distance, neighbour-joining method for initial tree building, maximum number of trees = 1000; Rzhetsky & Nei 1993) and maximum-parsimony (MP: heuristic search, close-neighbour-interchange method with the random addition of 1000 trees; Nei & Kumar 2000) trees were computed in MEGA 2.1. Robustness of nodes was confirmed by bootstrapping (1000 replicates). Two hamster species, *Cricetulus migratorius* and *Cricetulus griseus*, were incorporated as outgroup species. Fu's F_S (Fu 1997) test and pairwise mismatch distributions within populations (Rogers 1995) were chosen to detect recent population growth (ARLEQUIN 2.001; Schneider *et al*. 2000). Parametric bootstrapping (1000 replicates) was carried out to test whether mismatch patterns obtained fit with a sudden-expansion model (Schneider & Excoffier 1999). Relative rate tests were performed to detect potential rate variation between phylogenetic groups and between genes. We chose the two-cluster test option (Takezaki *et al*. 1995) as implemented in PHYLTEST 2.0 written by Kumar (Pennsylvania State University). The program allows the incorporation of multiple sequences into one lineage. Two lineages were then compared against a third outgroup. Level of incongruence between genes was tested with PAUP 4b5 (option Hompart). This approach uses the incongruence length difference (ILD) test with the parsimony criterion; 1000 randomizations were performed on variable sites only (Farris 1985); *C. migratorius* and *C. griseus* served as outgroups. For molecular clock analyses on mt haplotypes, we used a divergence rate of 7.5–13% as proposed by Galbreath & Cook (2004) for *Microtus oeconomus*. Their rates were applied to combined cyt *b* and *ctr* sequences and are based on divergence calculations obtained from two different arvicolid genera *Lemmus* (Fedorov & Stenseth 2001) and *Microtus* (Conroy & Cook 2000b).

Microsatellite analysis

Hamsters were genotyped at 11 microsatellite loci (Neumann & Jansman 2004). Mean number of alleles (*A*) and observed heterozygosity (H_O) were calculated in GENEPOP (Raymond & Rousset 1995). Rogers' genetic distance, *Dr* (Rogers 1972) between populations and population groups was computed in POPULATIONS (http://www.cnrs-gif.fr/pge/index.php?lang = en). Bootstrapping was carried out over the number of loci. The resulting tree was drawn in TREEVIEW (Page 1996). Allele size range, *R*, was measured as the sum of possible mutational steps deduced from overall allele distributions to account for unusual size mutants, which do not affect the actual size span.

Results

Table 2 provides diversity measures for mitochondrial and microsatellite loci as well as the number of individuals included in different analyses.

Mitochondrial data

Thirty-six *ctr* haplotypes (new haplotypes under AJ633722–38, GenBank) were found in 200 individuals. Twenty-nine sites proved variable among 337 bp of sequence, of which 16 mutations were parsimony informative. Only four transversions were observed. Two of them occurred in a single Russian haplotype (Mozdok, Caucasus).

Seventeen *16S* haplotypes (AJ633739–55) were identified in 130 animals. Sixteen out of 468 nucleotides proved variable and 11 were parsimony informative. Five of 18 mutations were transversions.

Twenty-seven cyt *b* haplotypes (AJ633756–82) were identified in 46 individuals. Nine hundred twenty-five (925) base pairs of sequence yielded 35 singletons and 27 parsimony-informative substitutions. Nine transversions were identified. Two haplotypes, Cb26 (Novosibirsk, Russia) and Cb27 (Brzezie, Poland), contained, in each case, two transversions. Fifteen mutations lead to amino acid changes.

The numbers of informative sites were either lower than (*ctr*, *16S*) or equal (cyt *b*) to haplotype numbers suggesting homoplasy. To enhance resolution, we combined *ctr* and *16S* in networks because both of these two DNA sequences were obtained from the largest numbers of individuals (n = 130). Parsimony as well as median-joining networks showed complex structures among haplotypes (for single genes as well as combined) as a consequence of recurrent mutations. Homoplasy was detected between German and Russian individuals and also inside Germany. Mutations

Table 2 Mitochondrial (N_H, haplotype number; π, nucleotide diversity) and microsatellite diversity measures of European common hamster phylogroups. Allelle number (*A*), size range (*R*) and observed heterozygosity (H_O) represent means over all loci. Microsatellite allele range is calculated as number of mutational steps and not as sequence length differences. Parameters of expansion (*Tau*/F_S) are based on pairwise mismatches of mt haplotypes

	Mitochondrial Loci			Microsatellite Loci		
Phylogroups (Sample ID)	N_H (*n*) *ctr* *16S* cyt *b* all combined	π ± SE	*Tau* (95% CI)/F_S	*A* ± SE (*n*)	*R* ± SE	H_O ± SE
West	5 (78)	0.5 ± 0.2	1.52 (0.00–2.97), *P* = 0.117/–4.29, *P* < 0.001	6.55 ± 1.00	5.9 ± 0.97	0.38 ± 0.06
(W1–W7)	2 (43)	0.2 ± 0.2	—	(137)		
	1 (10)	0	—			
	5 (10)	0.1 ± 0.1	3.00 (0.45–4.31), *P* = 0.560/–3.83, *P* = 0.001			
Central	13 (58)	0.9 ± 0.3	2.97 (0.94–4.36), *P* = 0.678/–12.92, *P* < 0.001	11.09 ± 1.46	9.8 ± 1.26	0.69 ± 0.04
(C1–C3)	8 (30)	0.4 ± 0.2	2.24 (0.21–3.76), *P* = 0.024/–7.42, *P* < 0.001	(148)		
	8 (12)	0.4 ± 0.1	2.73 (0.86–5.92), *P* = 0.972/–4.99, *P* < 0.001			
	7 (10)	0.3 ± 0.1	8.32 (4.10–12.99), *P* = 0.690/–5.16, *P* = 0.005			
North	16 (136)	0.9 ± 0.3	2.83 (0.76–4.06), *P* = 0.217/–18.61, *P* < 0.001	11.55 ± 1.53	10.5 ± 0.13	0.55 ± 0.04
(W1–7 + C1–3)	10 (73)	0.4 ± 0.2	2.43 (0.20–3.85), *P* = 0.024/–10.38, *P* < 0.001	(285)		
	9 (22)	0.4 ± 0.1	3.47 (1.22–5.60), *P* = 0.947/–6.06, *P* = 0.001			
	12 (20)	0.4 ± 0.1	8.32 (3.81–13.17), *P* = 0.700/–5.16, *P* = 0.006			
Pannonia	13 (44)	1.1 ± 0.3	3.70 (1.46–5.42), *P* = 0.784/–11.51, *P* < 0.001	13.27 ± 1.34	15.0 ± 1.81	0.76 ± 0.02
(P1, P2)	1 (40)	0	—	(105)		
	13 (18)	0.6 ± 0.2	7.74 (3.33–11.85), *P* = 0.537/–4.99, *P* < 0.001			
	10 (14)	0.5 ± 0.1	5.65 (2.40–14.58), *P* = 0.260/–3.87, *P* = 0.017			
E1–E7	7 (20)	1.1 ± 0.3		7.4 ± 0.45	9.4 ± 0.83	0.60 ± 0.04
	6 (17)	0.6 ± 0.2		(24)		
	5 (6)	1.6 ± 0.3				
	6 (12)	1.2 ± 0.2				
Total	36 (200)	1.5 ± 0.3	5.16 (2.90–6.57), *P* = 0.401/–25.57, *P* < 0.001	17.36 ± 2.79	16.8 ± 1.65	0.60 ± 0.03
	17 (130)	0.9 ± 0.3	2.20 (0.52–6.41), *P* = 0.008/–16.26, *P* < 0.001	(414)		
	27 (46)	1.2 ± 0.2	13.83 (8.14–18.02), *P* = 0.779/–19.33, *P* < 0.001			
	28 (46)	1.1 ± 0.2	26.43 (18.43–31.94), *P* = 0.26/–10.58, *P* < 0.001			

*Note that mean allele size ranges do not include *Ccrμ6* whereas other microsatellite measures are based on all 11 loci.

between affected haplotypes were down weighted in median-joining networks (Bandelt *et al*. 1999). Networks (Fig. 2, only the median-joining network based on combined *16S* + *ctr* sequences is shown) as well as gene trees (Fig. 3, only the ME tree based on all mt genes combined is shown) consistently separated western and central European hamsters into two well-defined lineages. Clade 'North' comprises all populations from Germany, France, Belgium and the Netherlands and therefore combines the original groups 'West' and 'Central' (see also Table 2). Hamsters from the Czech Republic and Hungary form the second clade 'Pannonia'. Both lineages do not share any mitochondrial haplotypes. Diversity values for cyt *b* and *ctr* between North and Pannonia were rather similar (Pannonia: $N_{Hctr} = 13$, $N_{Hcyt\ b} = 13$; $\pi_{ctr} = 1.1 \pm 0.3\%$, $\pi_{cyt\ b} = 0.6 \pm 0.2\%$ vs. North: $N_{Hctr} = 16$, $N_{Hcyt\ b} = 9$; $\pi_{ctr} = 0.9 \pm 0.3\%$, $\pi_{cyt\ b} = 0.4 \pm 0.1\%$). Pannonian hamsters proved invariant for *16S* unlike all other populations. D_a values between the two phylogeographical groups were as $D_{a\ ctr} = 1.0 \pm 0.4\%$, $D_{a\ 16S} = 1.3 \pm 0.5\%$, $D_{a\ cytb} = 0.9 \pm 0.3\%$, $D_{a\ comb.} = 1.1 \pm 0.3\%$.

Both main lineages show signs of further substructuring. The northern lineage is divided into highly polymorphic populations from central Germany (Central; C1–C3, $N_H = 8$–13) and less polymorphic western hamsters (West; W1–W7, $N_H = 1$–5). West appears highly bottlenecked, with very low π values (*ctr* = 0.5 ± 0.2%, *16S* = 0.2 ± 0.2%, cyt *b* = 0). Overlapping haplotypes between West and Central were restricted to the *ctr* sequences (Dl01, Dl07) but only the MP tree showed significant genetic divergence. All tree-making methods confirm a significant separation of Hungarian and Czech hamsters within the Pannonian lineage and show almost identical topologies. Haplotypes from Poland (E1) and Russia (E3–7) do not consistently cluster with any of the other groups (single genes). There is evidence for more than one phylogeographical lineage in the eastern sample (E3, E5 + E6, E7) although the existence of a single and very heterogeneous eastern phylogroup cannot fully be excluded. One well-supported clade comprises hamsters from Kirov (E5) and the Ural area (E6), which are close to the species' northern boundary. A

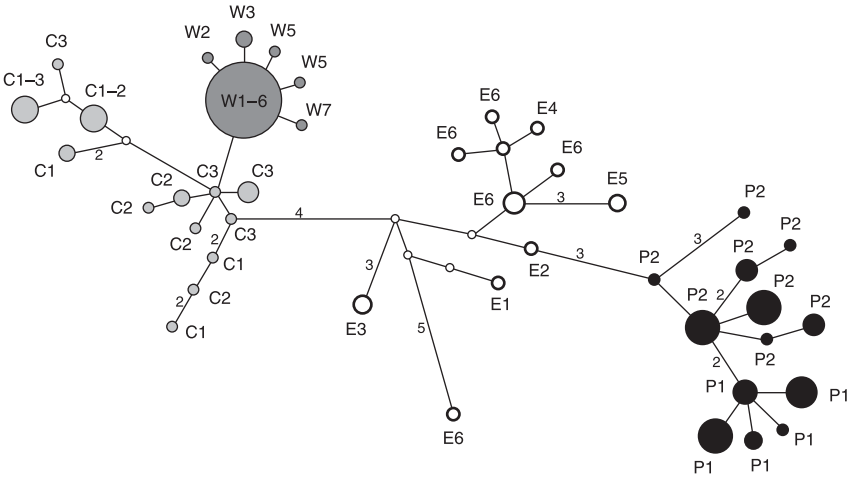

Fig. 2 Median-joining network based on combined *ctr* + *16S* haplotypes ($n = 43$) obtained from European common hamsters ($n = 130$). Small empty circles refer to missing intermediates with relevance for construction of links. Links were modified in cases where more than one connection was possible. Numbers on links refer to mutational steps dividing haplotypes. Geographic locations are indicated as shown in Table 1 and Fig. 1.

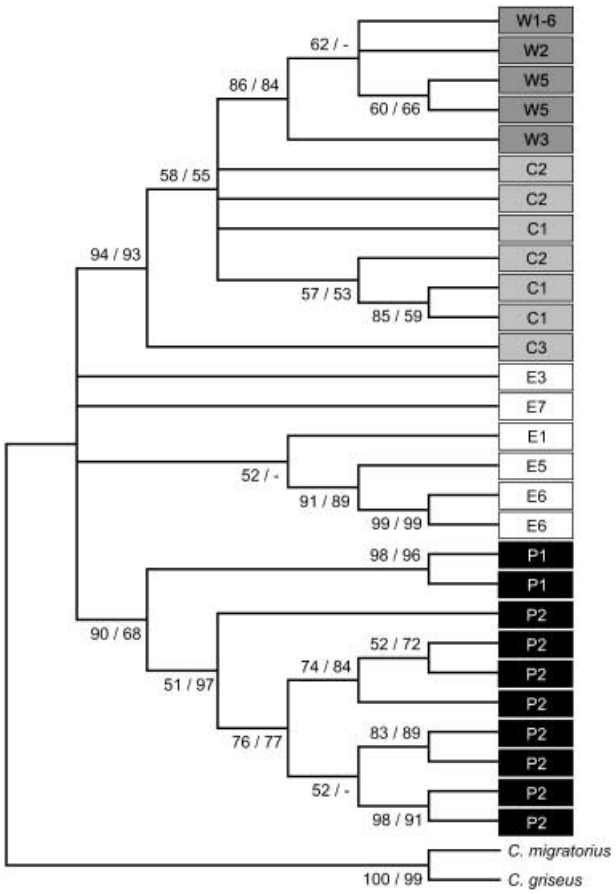

Fig. 3 Consensus ME tree based on 28 combined mt haplotypes (*ctr* + *16S* + cyt *b*) of 16 common hamster populations. Numbers on branches correspond to boostrap support (1000 replicates; ME/MP). Haplotypes of two related hamster species *Cricetulus griseus* and *Cricetulus migratorius* served as outgroups. Labels are as in Table 1.

Polish hamster associated with this group when the ME method was applied. According to the networks, Romania (E2) represents a link between Russian and Pannonian hamsters.

Pairwise mismatch analyses of mitochondrial genes revealed unimodal patterns for all population groups, congruent with a recent expansion/contraction scenario. Goodness-of-fit tests confirm the correctness of the mismatch distributions for all single gene analyses, except for *16S* in Central and North (both $P = 0.024$), and for combined genes in all cases. P values above 0.05 confirm sudden expansion. Fu's F_S rejects constant size for single genes ($P = 0.001$) and combined genes ($P = 0.017–0.001$).

The relative rate test indicated no significant rate heterogeneity between phylogeographical groups or between genes (*ctr*: $Z = 1.344$, *16S*: $Z = 1.344$, cyt *b*: $Z = 0.380$, all not significant) using the K2P distance. To carry out the test, we compared the groups North and Pannonia against a third group containing all eastern samples (E1–7). High congruence between mt genes ($P = 0.001$) was also proven by an ILD test. These results and the fact that all mt genes show similar π values for the entire sample ($\pi_{ctr} = 1.5 \pm 0.3\%$, $\pi_{16S} = 0.9 \pm 0.3\%$, $\pi_{\text{cyt } b} = 1.2 \pm 0.2$) allow their combination for time estimates. Based on that, we obtained the following molecular datings: 85 000–147 000 BP (95%CI: 39 000–225 000 years) for the split between North and Pannonia, 37 000–64 000 years (95%CI: 17 000–102 000 years) for the expansion of North and 25 000–44 000 years (95%CI: 11 000–112 000 years) for the expansion of Pannonia.

Microsatellites

DNA profiles of 414 individuals were obtained. The NJ tree has only limited resolution and a star-like topology (Fig. 4). The low bootstrap significance reflects the similar allele compositions of geographical groups. Two microsatellite loci, *Ccrμ3* and *Ccrμ6*, harbour unusual allele length variants. A 192-bp allele having an additional single nucleotide insertion is found in central German populations as well as across Hungary. A characteristic gap dividing small and large size alleles at the tetranucleotide locus *Ccrμ6* occurs

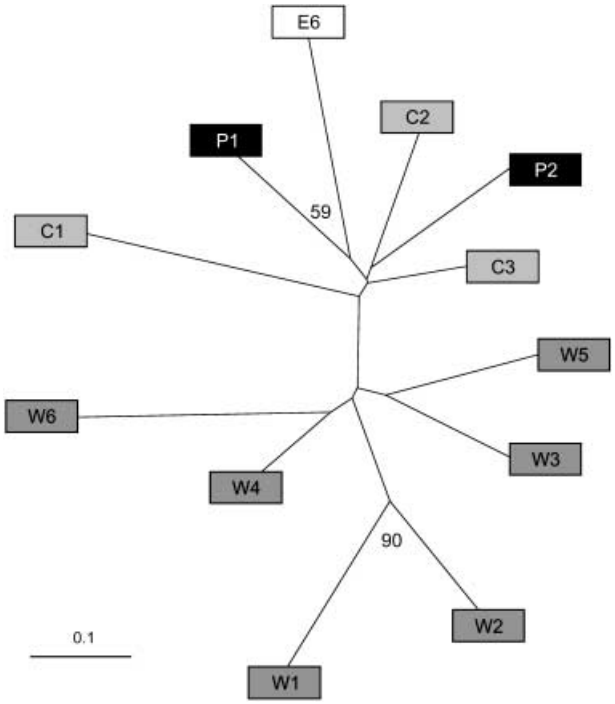

Fig. 4 Neighbour-joining tree based on 11 microsatellite loci comprising 12 European common hamster populations (Rogers' genetic distance). Numbers on branches represent bootstrap support (1000 replicates; based on number of loci). For labels see Table 1.

in Pannonia and Central, but large alleles with an extra GA insertion distinguish Pannonian and eastern hamsters.

Pannonia and Central exhibit highest observed heterozygosity values with $H_O = 0.76 \pm 0.02$ and 0.69 ± 0.04, respectively. West is much less heterozygous $H_O = 0.38 \pm 0.06$.

Pannonia and North exhibit high observed heterozygosity values with $H_O = 0.76 \pm 0.02$ and 0.55 ± 0.04, respectively. The heterozygosity of North is reduced by the low polymorphism of western hamsters (West: $H_O = 0.38 \pm 0.06$; Central: 0.69 ± 0.04). North shows a slightly smaller allele number ($A = 11.55 \pm 1.53$) and allele size range ($R = 10.5 \pm 0.13$) than Pannonia ($A = 13.27 \pm 1.34$; $R = 15 \pm 1.81$). Diversity of the single representative eastern population from the Urals (E6, $n = 19$) was $H_O = 0.61 \pm 0.04$, $A = 5.45 \pm 0.47$, which is comparable to the means of the entire eastern sample ($H_O = 0.60 \pm 0.04$, $A = 7.4 \pm 0.45$, $n = 24$).

Discussion

Glacial history and the timing of population differentiation

The phylogeographical pattern of European common hamsters shows the existence of two major allopatric mt lineages, Pannonia and North, as well as the presence of further lineages in Russia. The distinct north–south division between hamster populations in central Europe differs from the phylogeographical structure found in other European muroids (Jaarola & Searle 2002; Michaux *et al*. 2003). Expansion from different glacial refugia does best explain the observed spatial pattern. However, the bringing together of genetic and palaeontological data is slightly hampered by the differing opinions about taxonomic relationships within the genus *Cricetus (*Pradel 1981; Von Königswald 1981; Kowalski 2001) and general problems with the dating of fossil sites (Markova *et al*. 1995).

The possible persistence of the recent *Cricetus cricetus* form in different parts of central Europe during the latest glacial maximum (20 000–18 000 BP) has been discussed (Werth 1936; Storch 1974). A re-evaluation of fossils found in Germany, which could be determined as truly being *C. cricetus* (Kind 1987; Ziegler 1995), led to the conclusion that the species withdrew from the region and did not return before the end of the Weichselian (15 000–10 000 BP). Grulich (1987) stressed the point that the species could not have survived through the Last Glacial Maximum in Europe, because of the unsuitable palaeoclimatic and palaeogeographical conditions (Ložek 1973; Kahlke 1981). In contrast, Jánossy (1986) and Hir (1997) showed an almost uninterrupted record of *C. cricetus* for Hungary from the lower Weichselian (Subalyuk, *c.* 40 000 BP) onwards.

Our molecular clock estimates suggest that the separation between North and Pannonia occurred around 85 000–147 000 BP (95% CI: 39 000–225 000 years). This time window encompasses a period of major temperature shifts, including the entire Eem interglacial (*c.* 135 000–115 000 BP). Fossils predict a first appearance of *C. cricetus* in central Europe at the beginning of the Eemian (e.g. Rathgeber & Ziegler 2003). It is possible that westward expansion and subsequent habitat loss due to increasing woodland during warmer parts of the last interglacial caused extensive structuring among hamster populations. However, extreme cold and arid conditions in particular towards the end of the Saale-Riss glaciation (*c.* 250 000–135 000 BP) could have caused a similar diversification. Furthermore, the timing strongly implies that the separation of the extant hamster lineages had already occurred before their recolonization of central Europe.

Expansion times of 37 000–64 000 years (95% CI: 17 000–102 000 years) for North and 25 000–44 000 years (95% CI: 11 000–112 000 years) for Pannonia fall inside the Weichselian (115 000–10 000 BP). This finding is concordant with the repeated appearance of *C. cricetus*-like hamsters in Europe during colder periods of the Pleistocene (Kowalski 2001; Spitzenberger 2001). It is not surprising that hamsters extended their range following the formation of the open steppe habitats that are typical for moderate glacial intervals and cooler phases during interglacials (Nadachowski 1989; Probst 1999). The data suggest that common hamsters could cope well with cold climates and hence the glacial

maximum caused certainly a retreat, but did not significantly affect the population size of initially expanding populations. Interestingly, the expansion time for Pannonian hamsters is associated with the arrival of *C. cricetus* in Hungary during the Subalyukian substage (Janossy 1986). Fossils, and the high genetic diversity of recent populations, support an uninterrupted presence of common hamsters in Hungary over the last 40 000–50 000 years. Hamsters were pushed back from the western parts of the Carpathian basin by the last glacial advance, as documented for Austrian populations (Spitzenberger 2001), but probably survived in the Hungarian plains. The lack of variability at the *16S* locus in Pannonian hamsters is intriguing because this cannot simply be explained by a past bottleneck, as other mt genes were not affected. The conservation of *16S* in Pannonia proved significant relative to expectation (Fisher exact test, $P = 0.011$). However, it should be mentioned that the unexplained invariance does not substantially alter time estimates (e.g. expansion time of Pannonia calculated from cyt *b* + *ctr* only; 34 000–60 000 years, 95% CI: 16 000–154 000 years).

As already deduced from the divergence time estimate, the isolation in a Hungarian refugium did not significantly contribute to the genetic separation between North and Pannonia, which must have occurred earlier, most likely in eastern refugia. Additional evidence for this comes from Romanian mt haplotypes which are intermediate between those of Pannonian and Russian hamsters. Although we had only one Romanian sample from outside the Carpathian basin, its association with Pannonian and Russian animals is informative. It reflects a southern expansion route which was still used by Ukrainian hamsters during gradations in the 20th century (Calinescu 1931). The most important retreat areas presumably existed in the large southern Russian steppe zone, which represents the main distribution centre of the species (Niethammer & Krapp 1982; Nechay 2000). Markova *et al*. (1995) showed from fossil records that, at the end of the last glaciation, the Russian hamster range contracted to the west with the Urals forming an eastern boundary. Recolonization of Siberia and central Asia started from there at the end of the Valdai (Weichselian) epoch (15 000–10 000 BP). In a similar way, hamsters abandoning the western range during the last glacial advance may have returned from such an eastern refugium when the climate improved, establishing the northern lineage. A highly structured retreat zone formed by the Ukrainian and southern Russian plains may have served as a source of repeated population expansion to shift species boundaries throughout the entire Pleistocene (Fig. 1). This is supported by fossil records for that region over the last 130 000 years (Markova *et al*. 1995). The existence of further lineages in the Russian sample gives evidence for the heterogeneous structure of the eastern retreat area. The common hamster may therefore provide a phylogeographical pattern that differs from those previously found for other small mammals (Bilton *et al*. 1998; Jaarola & Searle 2002). However, Haynes *et al*. (2003) reported two main eastern European lineages in *Microtus arvalis*, another rodent adapted to open landscapes. More frequent spatial population movements along the northern boundary of the refugium could have led to recurrent acquisition of mt haplotypes explaining homoplasy in North but not in Pannonia. Evidence for expansion from a bottleneck (118 000–204 000 BP, 95% CI: 68 000–266 000 years) is found for the entire hamster sample. This may indicate that all extant *Cricetus* hamsters could have originated from a small population in the middle Pleistocene, perhaps during the penultimate glaciation.

Despite significant differences between mt haplotypes, there are obvious similarities at the nuclear level. High mutation rates in microsatellites may cause notable levels of homoplasy in distantly related populations (Estoup *et al*. 1995; Jarne & Lagoda 1996) explaining the lack of resolution when using allele frequencies to discriminate hamster populations. In contrast, a specific allele-spacing pattern at locus *Ccrμ6* and the unexpected sequence variant at locus *Ccrμ3* are very likely identical by descent. The 192-bp allele at locus *Ccrμ3*, found in central German and Hungarian hamsters, shows not only an additional nucleotide insertion but also three-point mutations not found in other alleles with regular dinucleotide variation (185, 191, 193, 195 bp). This accumulation of mutations identifies this particular allele as one which already has persisted for a long time in hamster populations. The evidence for sympatry that is seen at the nuclear level can be readily explained by the much larger effective population size compared to maternally inherited genes (Zhang & Hewitt 2003). The sharing of identical microsatellite alleles, as well as similar allele frequencies, suggests a relatively recent common ancestry of current hamster populations, complementing mt data in this respect.

Spatial structure in western and central Europe is maintained by geographical and ecological barriers

The contemporary phylogenetic structure of common hamsters in Europe is largely the result of expansion from a highly structured eastern refugium covering the Russian plains. Differentiation is enhanced by lineage sorting caused by further census size fluctuations, and the probable founder event which led to the division of North into subgroups Central and West. A core area for the expansions being within the Russian plains appears very likely, considering the fact that common hamsters represent typical continental steppe animals adapted to open landscapes. Suitable hamster habitats with mesic climates and deep loess soils are not widely distributed in central and western Europe leading to a disjunctive pattern of distribution. Therefore, historic and current partitioning of European lineages must be the

result of geographical and ecological barriers although anthropogenic influence allowed the colonization of previously uninhabitable areas. A very efficient north–south barrier is provided through a mountain chain formed by the Carpathians, Sudetes and German uplands isolating the two major central European lineages, North and Pannonia. Mountains generally play an important role for the impediment of dispersal in small mammals (Bilton *et al*. 1998). As a result, Pannonian hamsters became trapped inside the Carpathian basin. A similar differentiation of southern (Hungary, Slovakia) and northern (the Netherlands, Germany) populations in central Europe was also observed in *Microtus arvalis* (Haynes *et al*. 2003). The German uplands constitute not only the east–west barrier preventing gene flow between western German hamsters and Pannonia, but also shield central German hamsters from western hamsters. The subdivision of the northern lineage is the result of a western expansion by central German hamsters. Star-like topologies and low levels of mt and microsatellite variability arose from a founder event (Neumann *et al*. 2004) predicted for leading edge dispersal (Hewitt 1996). Once arrived, hamsters spread along the Rhine valley supported by increased farming in Neolithic times (Dupont 1932; Clason 1999). River valleys seem to represent important migration routes because of suitable microclimatic conditions and extensive agriculture. If German uplands efficiently interrupted gene flow, westward expansion was only possible at times of favourable climatic circumstances, allowing the animals to overcome lower mountains, or bypass them by a northern route. Ecological conditions had and still have an important influence on the spatial distribution of common hamsters. An invisible ecological frontier prevented the establishment of long lasting populations in the northern parts of Poland and Germany (Werth 1936; Surdacki 1971). Deteriorating climatic conditions may also have caused the separation from central German and eastern populations. Several studies show a wider western distribution of the common hamster during late glacial periods (summaries in Niethammer & Krapp 1982 and Spitzenberger 2001). It is noteworthy that the Polish sample shows no association with German animals and thus may have originated from a different wave of expansion that did not reach Germany. Until now, no contact zones were identified between any of the phylogenetic groups. However, hamster sites in Europe are highly fragmented and recent densities are low, reducing the chance of successful dispersal (Nechay 2000). Therefore, any contact zones presumably only existed temporarily.

Outlook

Fossil records and current genetic structure show a highly dynamic range development of the common hamster in Europe. Multiple wave-like expansion events are consistent with the species' high reproductive potential. It is noteworthy that familiar patterns of west–east and north–south divergence between phylogeographical groups are the result of expansion routes restrained by ecological and geographical barriers. Such barriers increase their significance when they fall close to range limits. The distribution of mtDNA phylogenetic lineages in central Europe does not correlate with the proposed existence of a western subspecies *Cricetus cricetus canescens* and an eastern form *Cricetus cricetus cricetus* (Mitchell-Jones *et al*. 1999) a finding which has implications for the conservation programs currently running. Our results also contradict a study by Smulders *et al*. (2003) that reported overlapping cytochrome *b* haplotypes between Dutch and Czech hamsters, but their study experienced some irregularities in the methodology (Smulders, personal communication).

The repeated range fluctuations during Pleistocene and past Holocene periods allow us to postulate the involvement of climatic factors in the large-scale negative population trends in common hamsters seen over the last 40 years. Dramatic declines of hamster populations in the Netherlands (Backbier & Gubbels 1998), Belgium (Mercelis 2002) and the extinction of northern populations in Germany (Krüger & Krüger 1998) show an ongoing withdrawal of the edges of the distribution. Spitzenberger (1998) reported that hamsters in Austria mainly retreated from areas with a colder and wet climate. Further studies should focus on the distribution and changes in the density of hamster populations in relation to climatic factors, for example winter humidity. Knowledge about historic range shifts may therefore provide valuable guidance. A more detailed analysis of the spatial genetic structure of eastern European common hamster populations is required to identify possible source populations for major central European lineages.

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