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White chest in the west: pelage colour and mitochondrial variation in the common hamster (*Cricetus cricetus*) across Europe

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Abstract The common hamster (*Cricetus cricetus* L.), a rodent of the Eurasian steppes and agricultural areas, is threatened by habitat loss. Remnant populations in Western and Central Europe are small, isolated and genetically impoverished. The populations of Belgium, The Netherlands and North Rhine-Westphalia, Germany (BNN), for which Nehring proposed the epithet *canescens*, are most affected by this decline. They are distinguished from more eastern populations by large, white areas on throat, chest and forelegs. These traits are sometimes also found in other populations, which casts doubt on their value as diagnostic characteristics. Here, we show that the frequency of occurrence of relatively large chest spots, chin streaks and cuffs on the forelegs is highest in BNN, where a white chest spot occurs in 67–100 % of the sampled individuals, compared to 0–8 % in Central and Eastern European populations. Additionally, hamsters from the Upper Rhine area also display relatively high frequencies of these characters (7–44 %). This suggests a common origin of BNN and Upper Rhine hamsters and an ancient expansion route along the Rhine Valley. A supplementary genetic study of two mitochondrial genes revealed extremely low diversity in both BNN and Upper Rhine hamsters but also clear differentiation and isolation between the two remaining relict populations of North Rhine-Westphalia.

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Introduction

The common hamster is a typical rodent of the European steppes and agricultural areas. Formerly widespread and common from Belgium to the Yenisei River in Siberia, hamster populations have declined dramatically since the 1950s and 1960s, and the species is now critically endangered in its western European range. The direct causes are not completely understood. Intensification of agricultural practices, such as early harvest and monocultures, which reduce plant cover and food availability in critical periods of the hamster's life cycle are considered the most important factors (Out et al. 2011). The resulting increased mortality is not compensated for by higher fertility. The common hamster has retreated from much of its former European range and the remaining habitat is highly fragmented by roads and urban areas.

The westernmost populations of the common hamster were affected most by this decline. The species went extinct in The Netherlands in 2002, although a re-introduction programme was started the same year (La Haye et al. 2010). Populations in Belgium and the Alsace (France) remain critically endangered, and the hamster's range in North Rhine-Westphalia (Germany) is reduced to two relict populations near Zülpich and Rommerskirchen (Fig. 1).

Hamster populations in The Netherlands, Belgium, France, as well as in the German states of North Rhine-Westphalia, Rhineland-Palatinate, Hesse, Baden-Württemberg and Bavaria, have often been regarded as belonging to the sub-species *C. c. canescens* (Nehring 1899), which was supposed to differ from the central German nominate form by smaller size and more greyish pelage. Curiously, Nehring did not mention a white chest spot in his description, which was based on

Fig. 1 Sampled populations for fur trait data (compare Table 1) and distribution of the common hamster. **a** Current (ca. 1990–2010, *black*) and former (ca. 1930–1960, *grey*) autochthonous distribution in Germany. *Light grey* denotes areas where the species was found only sporadically due to sub-optimal environmental conditions. Even in the areas marked in *black*, populations are now highly fragmented by roads and settlements and do not form continuous distributions. **b** Former range of the common hamster in Europe. Using data from Werth (1934); Niethammer (1982); Seluga (1998); Nechay (2000); Endres (2004); Rietschel and Weinhold (2005); Weinhold and Kayser (2006); Hellwig (2009); Lux and Görner (2009); Meyer (2009); Schreiber (2010); Vohralík (2011); Gall and Jokisch (2011); and Eidenschenk and Grandadam (2012)

two stuffed skins from Fexhe-Slins, Belgium. These syntypes could not be traced in the Berlin Museum, but all remaining Belgian skins possess conspicuous chest spots. Wepner (1936) dismissed size differences as more dependent on age, but proposed a white chest spot (Fig. 2) as a defining characteristic of *canescens*, which, according to her, included all hamsters west of the Rhine. Husson (1959) found several traits that were more pronounced in *canescens*, such as the frequency and size of white-coloured areas between the ears, on throat, chest and forelegs, and cream-coloured thigh spots. He assumed that the white fur traits were dominant and spreading in central German populations. Husson also proposed an index of the length of the foramen incisivum and the length of the posterior part of the palate, which was supposed to be the highest in *canescens*. Other authors rejected the subspecies status of *canescens* (Novikov 1935; Niethammer 1982) based on inadequacies in Nehring's description and the fact that hamsters from Rhenish Hesse show intermediate characteristics between Belgian and Dutch, and central German hamsters, and the occasional occurrence of chest spots in eastern populations. Stefen (2013) found cranial variations to be insufficient to clearly separate *canescens* as a sub-species based on skull measurements. A low genetic diversity was found in *canescens* populations for both mitochondrial and nuclear genes (Neumann et al. 2005; La Haye et al. 2011), which was attributed to re-colonisation founder effects and the recent decline.

Molecular studies by Neumann et al. (2004, 2005) recovered several distinct phylogeographic lineages in Europe. The North lineage comprises the Belgian, Dutch, French and German hamsters. It can be divided into a Central group of central and east German hamsters and a West group corresponding to the proposed *canescens* sub-species. The West group is, in turn, composed of populations of Belgium, The Netherlands, and North Rhine-Westphalia (BNN) (La Haye et al. 2011) and the Upper Rhine populations in Rhineland-Palatinate, Hesse, Baden-Württemberg, Bavaria and Alsace in France. The Pannonian Basin is inhabited by the Pannonia lineage, which has also spread into southern Poland (Banaszek et al. 2010). The East group in Eastern Europe likely does not constitute a true phylogroup, but rather several lineages (Neumann et al. 2005). Its western distribution maximum lies in eastern Poland (Banaszek et al. 2010).

The aim of this study is to assess the occurrence of morphometric traits attributed to *canescens* and the West phylogroup in all European populations to test its separate status and to reconstruct the relationship between these populations. Since the German populations of North Rhine-Westphalia and Rhineland-Palatinate are, so far, underrepresented in molecular studies, a second goal was to compare their mitochondrial haplotypes with better-known populations.

Material and methods

A total of 626 skins were examined from the following collections: Zoologisches Forschungsmuseum Alexander Koenig Bonn (ZFMK, 236), Museum für Naturkunde Berlin (ZMB, 160), Naturmuseum Senckenberg Frankfurt am Main (SMF, 130), Naturalis Leiden (RMNH and ZMA, 94) and Musée zoologique de la ville de Strasbourg (6). Specimens were assigned to established phylogeographic lineages (Neumann et al. 2004, 2005) and sub-lineages based

Fig. 2 Pelage colour variations in the common hamster. **a** ZMFK_MAM_2012.327 from Rommerskirchen (North Rhine-Westphalia) with pronounced *white areas* on the ventral side. **b** ZFMK_MAM_2008.172 from Eimsheim (Rhineland-Palatinate). In this specimen, the chest spot is absent and the cuffs and chin streak are short. The *light area* on the throat is caused by hair loss. **c** ZFMK_MAM_2008.130 from Eimsheim with two conspicuous *cream-coloured thigh spots* (*arrows*). **d** ZFMK_MAM_2008.132 from Eimsheim. In this specimen, the thigh spots are completely absent
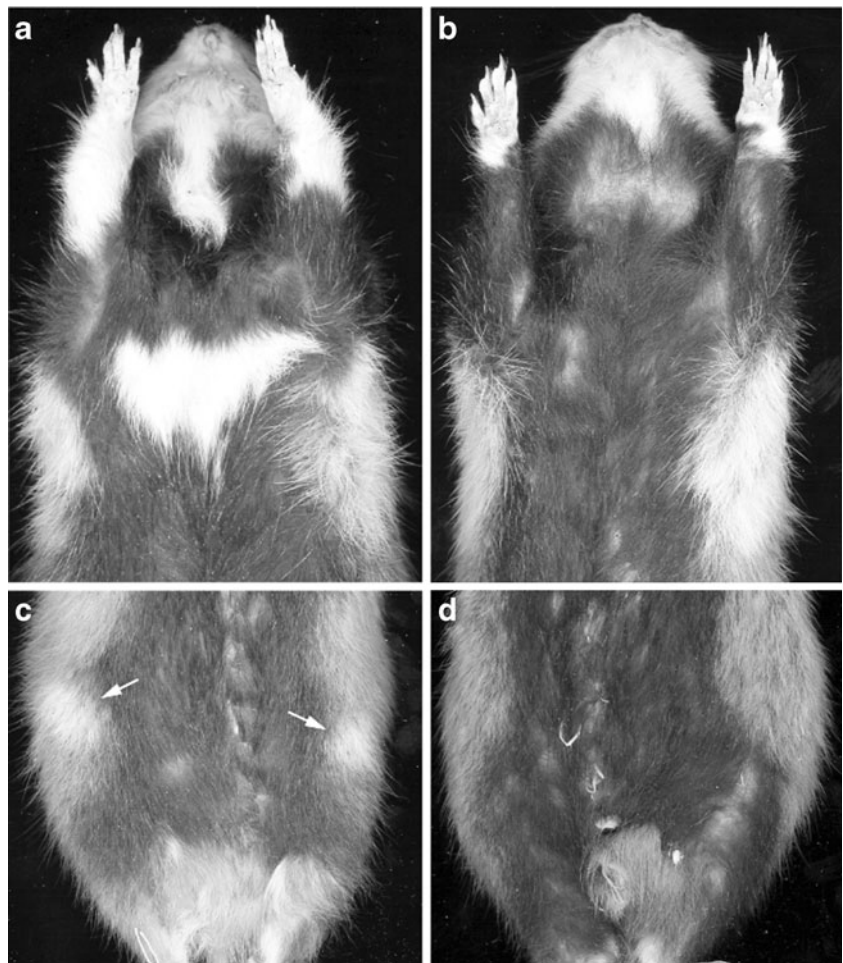
on locality as follows: BNN (148) with Belgium (4), The Netherlands (86) and North Rhine-Westphalia (59); Upper Rhine (297) with Rhineland-Palatinate (219), Hesse (59), Baden-Württemberg (13) and Alsace (6); Central (97) with Lower Saxony (7), Saxony-Anhalt (33), Thuringia (6), Saxony (5), Brandenburg (17), Mecklenburg-Western Pomerania (4) and western Poland (25); Pannonia (64) with Austria (8), Hungary (34), Slovakia (1), Croatia (2) and western Romania (19); and Eastern (20) with eastern Romania (4), Belarus (1), Ukraine (2) and Russia (13). These specimens were collected between 1872 and 2006.

Four fur traits were measured on the skins as follows: Chest Spot length (anteroposterior dimension of the ventral chest spot), Chest Spot width (left–right dimension), Chin Streak length (from lower lip to posterior margin) and Cuff length (from proximal end of pad to proximal margin) (Fig. 3f). Measurements were taken to the nearest millimetre. Additionally, the presence of chest spots and thigh spots was recorded in each skin. In some individuals, diffuse white hairs were present on the chest without forming a distinct spot. Snout–vent length was recorded from the collection label if possible; otherwise, it was measured on the skin to

the nearest centimetre. Specimens with other colour variations, such as melanism or flavism, were not included. No significant differences between sexes were found for the traits, so they were pooled.

Three indices were calculated from the data to compare relative sizes of the white fur traits as follows:

$$\text{SpotI} = \frac{100 \times \sqrt{\text{chest spot width} \times \text{chest spot length}}}{\text{SVL}}$$

$$\text{StreakI} = \frac{100 \times \text{streak length}}{\text{SVL}}$$

$$\text{CuffI} = \frac{100 \times \text{left cuff length}}{\text{SVL}}.$$

All data were tested for normality using the Shapiro–Wilk test. Since the data were not normally distributed, Mann–Whitney U was used for pairwise tests. A Kruskal–Wallis test was used to test groups, and Spearman's Rho to test
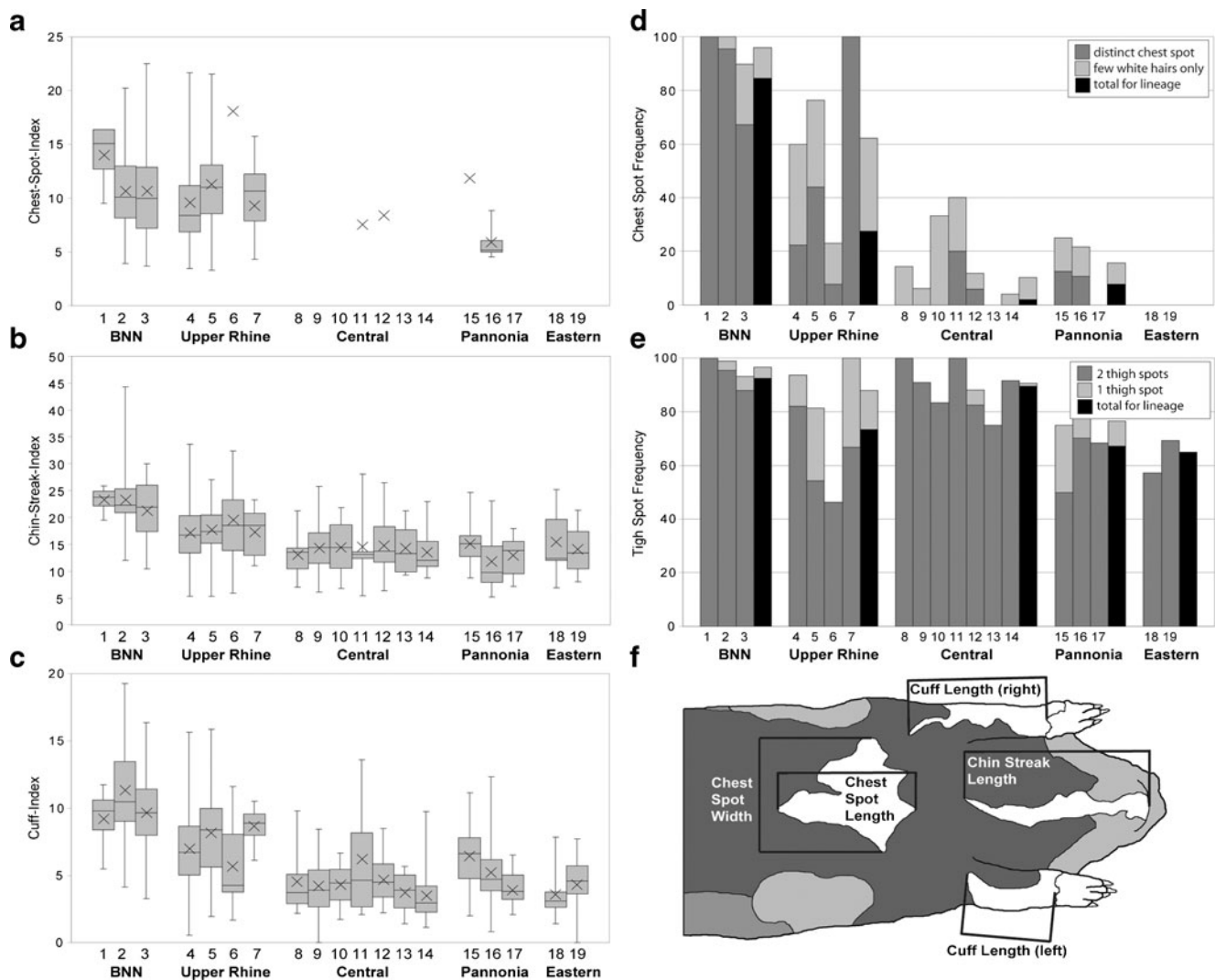
Fig. 3 *Box plots* of the relative sizes of the three white fur traits as follows: **a** chest spot size, **b** chin streak length, and **c** cuff length, as well as frequencies of **d** the white chest spot, and **e** the cream-coloured thigh spots in hamsters from different European populations. A clear trend from western to eastern populations is visible. **f** The measurements taken. *Numbers* on the *X*-axes denote sampled populations (compare Table 1). "Eastern" does not denote a true monophyletic lineage, but rather a wastebasket group of East European and Asian specimens

correlation. All statistical tests were done in PAST (Hammer et al. 2001).

Tissue samples were taken from a total of 48 hamsters. Eighteen hamsters were collected in the two relict populations of North Rhine-Westphalia by members of the Naturschutzbund Deutschland (NABU) in 2003–2006. All of these animals were either found dead, died in captivity or were killed by domestic cats. An additional 30 specimens from the collection of the ZFMK, collected from 1978 to 1999 in North Rhine-Westphalia, Rhineland-Palatinate and Austria, were sampled. If available, samples were taken from muscle tissue. In some cases, dried skin from mummified remains was taken instead, which also yielded good results.

Two partial mitochondrial genes were investigated as follows: the first sub-unit of cytochrome *c* oxidase (COI) using the 'barcoding' primer cocktail C_VF1LFt1–C_VR1LRt1 (Ivanova

et al. 2007) and part of the displacement loop (D-loop) of the control region (ctr) using the primers DlInt1 and DlInt2 designed by Neumann et al. (2005).

DNA was extracted from the tissue samples using the BioSprint96 magnetic bead extractor by Qiagen (Hilden, Germany). Polymerase chain reaction was carried out in total reaction mixes of 20 μl, including 1.2–2 μl of undiluted DNA template, 1.6 μl of each primer (10 pmol/μl), 2 μl of 'Q-Solution' and 9.5 μl of 'Multiplex PCR Master Mix', containing hot start Taq DNA polymerase and buffers. The latter components are available in the Multiplex PCR kit from Qiagen (Hilden, Germany). PCR reactions were run individually and not multiplexed. Thermal cycling was performed on GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA) as follows: hot start Taq activation: 15 min at 95 °C; first cycle set (15 repeats):

35-s denaturation at 94 °C, 90-s annealing at 60 °C (−1 °C/cycle) and 90-s extension at 72 °C. Second cycle set (25 repeats): 35-s denaturation at 94 °C, 90-s annealing at 50 °C and 90-s extension at 72 °C. After enzymatic cleanup using ExoSAP-IT, PCR products were sent to a sequencing facility (Macrogen, Amsterdam, NL). All sequences were submitted to GenBank under accession numbers KC953754–KC953838.

Haplotype diversity and nucleotide diversity were calculated in DnaSP 5.10.01 (Librado and Rozas 2009). A statistical parsimony network of the haplotypes was constructed using TCS 1.21 (Clement et al. 2000).

Results

The sampled populations show clear geographical variation in the distribution of the examined fur colour traits. The occurrence of extended white areas on the throat, chest and forelegs and cream-coloured thigh spots is most prevalent in the westernmost populations, while in the more eastern populations, these traits are far less frequent and pronounced (Fig. 3, measurements in the Supplementary material tables S1–3). The snout–vent lengths of the BNN and Upper Rhine populations are also significantly smaller ($p < 0.01$) than those of the more eastern lineages; however, the individual ages of the hamsters were not estimated.

The BNN and Upper Rhine lineages were shown to differ significantly from all other lineages, respectively, for all fur traits, except the relative size of the chest spot (SpotI) and the frequency of thigh spots between BNN and Central populations. Between the remaining lineages, generally only the frequency of the thigh spots proved to differ significantly (Table 1).

According to Spearman's Rho, the relative sizes of the three white fur traits are highly significantly correlated ($p < 0.001$) across all populations, with a correlation coefficient of $r_s = 0.29$ for SpotI and StreakI, $r_s = 0.33$ for SpotI and CuffI and $r_s = 0.48$ for StreakI and CuffI. In all sampled populations the specimens were collected mostly contemporarily to each other and little modern material is available, so that no trends over time could be reconstructed. No significant differences between sexes could be found for any of the examined traits.

Of the mitochondrial control region (ctr), a fragment 212 bp in length was sequenced (compare 337 bp in Neumann et al. 2004, 2005) in 46 hamsters (GenBank KC953754–KC953799). Eight nucleotide positions proved variable, six of which are parsimony informative while two are singletons. Four ctr haplotypes were identified in the sample, three of which (CCdl01, CCdl10 and CCdl24) had been reported previously (GenBank AJ550189, AJ550198 and AJ633726; Neumann et al. 2004, 2005). A novel ctr haplotype was found in ZFMK_MAM_99.1260 (GenBank KC953774) from Frauenkirchen, Austria. All hamsters from North Rhine-Westphalia were fixed for CCdl01. This was also the most common haplotype of Rhineland-Palatinate samples, but CCdl10, a haplotype previously known from Thuringia, occurred in an animal from Eimsheim (ZFMK_MAM_2008.114; GenBank KC953794) (Table 2). Of the three Austrian samples, two possessed the haplotype CCdl24, previously known from Hungary, while the novel haplotype CCdlAu01 occurred once.

Five previously unknown COI haplotypes of 665-bp length were sequenced in 39 hamsters (GenBank KC953800–KC953838). Thirteen positions were variable, with 12 parsimony-informative sites and one singleton. In the animals from North Rhine-Westphalia, the two relict populations each possessed a different haplotype. All animals from the Rommerskirchen area were fixed for CcCOI01, while the Zülpich animals were fixed for CcCOI02. Most animals from Rhineland-Palatinate shared CcCOI01, but CcCOI03 occurred in a single specimen from Eimsheim (ZFMK_MAM_2008.121; GenBank KC953834). CcCOI04 and CcCOI05 were found in Austrian hamsters.

Haplotype diversities H for ctr and COI are low in RP at 0.095 and 0.01, respectively, owing to the predominance of a single haplotype. Haplotype diversity for COI in NRW is calculated as 0.529, but this value is not meaningful, since the two relict populations that are fixed for one haplotype each are completely isolated. Austrian haplotype diversity reaches 1, since only two samples with different haplotypes yielded positive results.

Nucleotide diversity π values for both ctr and COI are very low in NRW and RP (<0.1 %), since the only differences between the haplotypes are single transitions. Austrian samples, even though only two sequences were recovered, show diversities of 0.31 % for ctr and 0.23 % for COI.

Discussion

This study supplements previous work on fur trait data by Husson (1959). A far larger sample of common hamsters was examined to find trends and differences in colouration between European populations. The results mirror his conclusions that the white fur traits are most pronounced in the westernmost parts of the hamster's range. This is especially apparent in the white chest spot, which occurs only rarely in the Central and Pannonia lineages, while more than 80 % of BNN animals possess a distinct spot. The chin streak and cuffs are also significantly longer in BNN and Upper Rhine populations, although the size of the chest spot, if present, did not differ significantly between populations. Definitive differences for the fur traits can only be shown for large sample sizes due to high variability between individuals. Grulich (1987) observed the same for skull measurements.

Table 1 Significant differences between phylogeographic lineages with their sampled populations for frequencies and size indices of the fur traits based on Mann–Whitney *U*. SpotF, chest spot frequency; SpotI, chest spot index; StreakI, chin streak index; CuffI, cuff index; ThighF, thigh spot frequency

		BNN	Upper Rhine	Central	Pannonia
BNN	*n*=148				
1. Belgium	*n*=4				
2. The Netherlands	*n*=86				
3. North Rhine-Westphalia	*n*=59				
Upper Rhine	*n*=297	SpotF ($p<0.001$)			
4. Rhineland-Palatinate	*n*=219	StreakI ($p<0.001$)			
5. Hesse	*n*=59	CuffI ($p<0.001$)			
6. Baden-Württemberg	*n*=13	ThighF ($p<0.001$)			
7. Alsace	*n*=6				
Central	*n*=97	SpotF ($p<0.001$)	SpotF ($p<0.001$)		
8. Lower Saxony	*n*=7	StreakI ($p<0.001$)	StreakI ($p<0.001$)		
9. Saxony-Anhalt	*n*=33	CuffI ($p<0.001$)	CuffI ($p<0.001$)		
10. Thuringia	*n*=6		ThighF ($p<0.001$)		
11. Saxony	*n*=5				
12. Brandenburg	*n*=17				
13. Mecklenburg-Western Pomerania	*n*=4				
14. Western Poland	*n*=25				
Pannonia	*n*=64	SpotF ($p<0.001$)	SpotF ($p<0.001$)	StreakI ($p<0.05$)	
15. Austria	*n*=8	SpotI ($p<0.05$)	StreakI ($p<0.001$)	CuffI ($p<0.01$)	
16. Slovakia/Hungary/Croatia	*n*=37	StreakI ($p<0.001$)	CuffI ($p<0.001$)	ThighF ($p<0.001$)	
17. Romania (west of Carpathians)	*n*=19	CuffI ($p<0.001$)	ThighF ($p<0.001$)		
		ThighF ($p<0.001$)			
Eastern	*n*=20	SpotF ($p<0.001$)	SpotF ($p<0.001$)	ThighF ($p<0.001$)	n.s.
18. Eastern Romania/Ukraine/Belarus	*n*=7	StreakI ($p<0.001$)	StreakI ($p<0.05$)		
19. Russia	*n*=13	CuffI ($p<0.001$)	CuffI ($p<0.001$)		
		ThighF ($p<0.001$)	ThighF ($p<0.001$)		

ns not significant

The three examined white-coloured traits show a strong positive correlation with each other, hinting at a common genetic pathway. The pattern of the white areas on the throat, chest and forelegs, as well as between the ears of some individuals (Husson 1959), is reminiscent of recessive spotting (piebald) in common voles (*Microtus arvalis*). In those voles, the chin and chest and a blaze on the head are the most common areas to be affected by pigment loss and, except for the toes and base of the tail in some individuals, only the front half of the animal is ever affected. White chin spots and stripes are also common in other *Microtus* and *Clethrionomys* species (Frank and Zimmermann 1957). In *Mus musculus*, a similar phenotype is caused by a delay in melanocyte migration from the neural crest. The affected areas on the chest and forehead are the last to be reached by melanocytes during embryonic development and, due to the delay the epidermis in these areas, is already differentiated enough to make their passing impossible (Danneel and Schumann 1961). According to breeding experiments conducted by Petzsch (1941), the white chest spot in hamsters is caused by an autosomal dominant allele. Some melanistic specimens (not included in the statistics) possess the examined white fur traits, as well as the normally white areas around the mouth and ears (cf. Petzsch 1941), while none of them show cream-coloured thigh spots. Since the white hairs of the lips mostly lack pigment (Kourist 1957), the same is likely true for the white hairs of the chest spot and chin streaks. An increased melanin production would, thus, not affect the extent of areas that lack pigment altogether.

The distribution of the white fur traits follows a somewhat clinal gradient, though the extant populations are no longer in contact. The longest chin streaks and cuffs are found in the Belgian and Dutch populations, while they are less pronounced in North Rhine-Westphalia. The Upper Rhine populations are intermediate for these traits between the BNN and Central hamsters. Except for the small Alsacian sample, chest spots and long streaks and cuffs are not as common as in the BNN group. The high frequency of long streaks and cuffs in Hesse particularly shows that the Rhine cannot form a barrier separating West from Central hamsters in Thuringia and Lower Saxony. Instead, the Central German Uplands, particularly the Thuringian Forest, Vogelsberg, Rhön and Spessart mountains, form the northern distribution border and act as barriers between the West and Central phylogeographic lineages. These barriers could only be crossed during favourable climatic conditions, when steppe habitats were more common, as was the case during several stages of the glacial and interglacial cycles (Neumann et al. 2005). Early forms of *Cricetus cricetus* and related forms such as *C. major* were widespread in Europe for most of the Pleistocene, apparently

Table 2 Haplotype frequencies, haplotype diversity H, nucleotide diversity π, and their standard deviations of the sampled populations for ctr, COI, and combined haplotypes. Not all amplifications yielded positive results

Locality	Number	Date of collection	Lineage[a]	Haplotypes (absolute frequency; relative frequency) ctr	H	π (%)	COI	H	π (%)	Combined H	π (%)
North Rhine-Westphalia	23		W1, W4	CCdl01 (22; 1.0)			CcCOI01 (8; 0.47) CcCOI02 (9; 0.53)	0.529±0.045	0.083±0.07	0.525±0.055	0.062±0.006
Tüddern (Dutch border)	1	1987	W1	CCdl01 (1; 1.0)			–				
Rommerskirchen group											
Rommerskirchen	10	2003–2005	W4	CCdl01 (9; 1.0)			CcCOI01 (8; 1.0)				
Frechen	2	1978, 1980	W4	CCdl01 (2; 1.0)			–				
Zülpich group											
Zülpich	8	2004–2006	W4	CCdl01 (8; 1.0)			CcCOI02 (8; 1.0)				
Essig (Swisttal)	1	1978	W4	CCdl01 (1; 1.0)			–				
Palmersheim (Euskirchen)	1	1983	W4	CCdl01 (1; 1.0)			CcCOI02 (1; 1.0)				
Rhineland-Palatinate	22	1986	W6	CCdl01 (20; 0.95) CCdl10 (1; 0.05)	0.095±0.084	0.045±0.040	CcCOI01 (19; 1.0) CcCOI03 (1; 0.05)	0.100±0.088	0.015±0.014	0.205±0.119	0.025±0.015
Einsheim	19	1986	W6	CCdl01 (17; 0.95) CCdl10 (1; 0.05)	0.111±0.096	0.053±0.046	CcCOI01 (17; 0.95) CcCOI03 (1; 0.05)	0.111±0.096	0.017±0.014	0.228±0.129	0.027±0.016
Hackenheim	2	1999	W6	CCdl01 (2; 1.0)			CcCOI01 (2; 1.0)				
Roxheim	1	1981	W6	CCdl01 (1; 1.0)			–				
Total Western	45				0.047±0.044	0.022±0.021		0.419±0.076	0.067±0.013	0.476±0.077	0.060±0.011
Austria	3	1984	P2	CCdl24 (2; 0.7) CCdlAu01 (1; 0.3)	0.667±0.314	0.314±0.148	CcCOI04 (1; 1.0) CcCOI05 (1; 1.0)	1±0.5	0.150±0.075	1±0.5	0.228±0.114
Bruck an der Leitha	1	1984	P2	CCdl24 (1; 1.0)			–				
Neusiedl am See	2	1990, 1994	P2	CCdl24 (1; 0.5) CCdlAu01 (1; 0.5)	1±0.5	0.472±0.236	CcCOI04 (1; 1.0) CcCOI05 (1; 1.0)	1±0.5	0.150±0.075	1±0.5	0.228±0.114
Total	48				0.167±0.073	0.399±0.194		0.478±0.079	0.243±0.113	0.532±0.078	0.279±0.139

[a] According to Neumann et al. (2005)

ranging from England to Israel (Niethammer 1982). Fossils were found in cave systems of the Eifel, Taunus and Harz mountains (compare Fig. 4) as well as sediments of the Bavarian Danube (Werth 1934). According to Grulich (1987), the Upper Würmian and Weichselian glaciation forced the hamster to retreat from most of its European range, and probably, it was only re-colonised during the postglacial climatic optimum at the end of the Atlantic. However, the Pannonian Basin seems to have acted as a refuge during this time, and Neumann et al. (2005) showed that the split between the Pannonia and North lineages may date back to 85,000–147,000 BP, surmising that both lineages separated before the Weichselian glaciation and that both had different eastern refugia. Their molecular dating also calculated a period from 37,000 to 64,000 BP for the expansion of the North lineage, to which the West hamsters belong. Since the BNN and Upper Rhine groups are situated at the extreme periphery of this expansion, the re-colonisation of the Rhine Valley probably occurred relatively late.

La Haye et al. (2011) suggested Zülpich, North Rhine-Westphalia, as a possible location for the crossing of the Rhine. They based this on a STRUCTURE analysis in which the Zülpich population formed a distinct genetic cluster and, according to them, forms a geographical link between BNN and Central hamsters. However, this study did not include any samples from Upper Rhine populations, which would very likely have clustered closer to these samples than to Central specimens. Furthermore, no large populations are known from the left bank of the river in North Rhine-Westphalia in historical times, since the habitat is unfavourable for hamsters (Werth 1934).
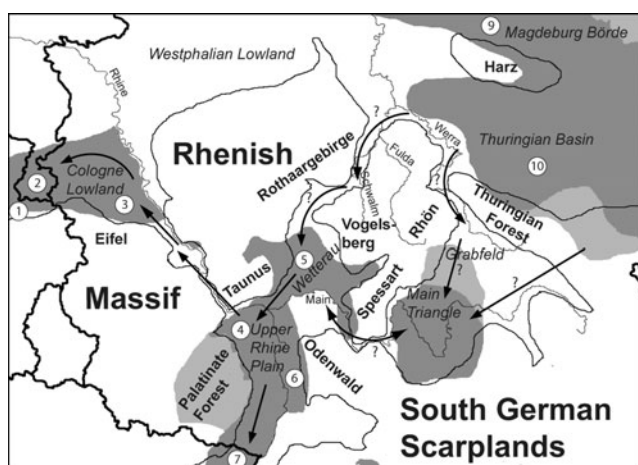
Fig. 4 Major upland (*bold*), and lowland (*italic*) areas, rivers (*regular*), and possible expansion routes (*arrows*) in western central Germany. The results of this study favour a colonisation of the Cologne Lowland via the Rhine Valley from the Upper Rhine Plain. Several possible routes are proposed for the colonisation of the Upper Rhine. Former distributions and numbers as in Figs. 1 and 3. The relations of the Lower Franconian population in the Main Triangle are unclear due to insufficient data

The situation is different further upstream. The populations of Hesse, east of the river and Rhineland-Palatinate west of the Rhine, are similar in their fur traits. The fact that the Upper Rhine populations are also intermediate in the fur trait frequencies between the BNN and the nominate form of central Germany establishes the possibility that all West hamsters descend from animals in the Upper Rhine area. These ancestors could have invaded the Wetterau from the Thuringian Basin, following the courses of the rivers Fulda and Schwalm (Fig. 4), resulting in a first founder effect that in all likelihood led to the higher frequencies of the white fur traits that we observed. The crossing of the Rhine later occurred in the northern Upper Rhine Plain, with the common hamster then spreading into Rhineland-Palatinate, Baden-Württemberg and Alsace. It is likely, though, that the river was not crossed just once, but that the populations of both banks were in contact with each other. In historic times, the Upper Rhine did meander extensively, forming many islands and shallow zones, before being artificially straightened in the seventeenth and eighteenth centuries. Thus, it is possible that the Upper Rhine was not a significant barrier for the expansion and exchange of the common hamster for most of its history.

From the Upper Rhine Plain, another expansion route followed the river's course northwards, thus crossing the Rhenish Massif and reaching the Cologne Lowland. This probably led to a second founder effect, which might explain the even higher frequencies of the fur traits in the resulting populations. This route is further supported by the fact that hamsters historically occurred on the west bank of the Rhine Valley, in the small Middle Rhine Basin within the Rhenish Massif (ZMB_MAM_32366, collected in Wolken near Koblenz; Werth 1934), and possibly also on the east bank (Hellwig 2009). From there, hamsters migrated farther into Dutch Limburg and Belgium, establishing the BNN lineage wherever soil and climatic conditions were favourable. According to Neumann et al. (2005), river valleys constitute favourable migration routes due to suitable microclimatic conditions and possible early agriculture. An expansion route via the Westphalian Lowland is unlikely, since climatic and soil conditions are unfavourable and common hamsters were never recorded there (Werth 1934). Additionally, a direct colonisation of the Cologne Basin from central Germany would not explain the intermediate status of the Upper Rhine populations in regard to the white fur traits.

Another possible expansion route is via Lower Franconia, where the hamster still occurs around the Main triangle and formerly the Grabfeld. By following the Werra River (Fig. 4) Thuringian hamsters could have reached this fertile area and a second wave following the Main downstream might have founded the Upper Rhine populations. On the other hand, Upper Rhine hamsters could have followed the Main

upstream, so that the Lower Franconian population descended from West stock. Unfortunately, sufficient morphological samples from Lower Franconia were not available for this study, so that their relationship within the North phylogroup could not be determined. Photographs of two skins from that area (Zoologische Staatssammlung München) show relatively short chin streaks and cuffs, and no chest spots. Whether the extinct population that formerly extended along the Danube River west of Ulm (Werth 1934) was also colonised by the West lineage has to remain unanswered. An interesting possibility is an expansion of the Pannonia lineage following the course of the river upstream. Unfortunately, no samples from this location were available and probably do not exist in any collection.

Husson (1959) noted the possibility that the examined white fur traits were spreading and becoming more common in Germany. The two Central specimens that possessed chest spots were collected in 1950, while most other specimens came from the 1910s. This pattern is contradicted by the closely related Hessian (mostly from the 1970s) and Rhineland-Palatinate (mostly from the 1980s) samples, where the Hessian specimens unexpectedly had a far higher frequency of chest spots and other pronounced fur traits. Unfortunately, for all sampled populations, the majority of specimens were collected contemporarily to each other and few modern samples are available, so that no clear progressions over time could be reconstructed. Larger current samples from all populations would be required to accurately assess the present situation of the fur traits.

The low genetic diversity in the hamster populations of Belgium and The Netherlands has been discussed previously (Smulders et al. 2003; Neumann et al. 2004; La Haye et al. 2011). For the first time, a large sample from North Rhine-Westphalia and Rhineland-Palatinate was covered in this study. As expected, analysis of two mitochondrial genes revealed that diversity in these populations is low, with North Rhine-Westphalian relict populations being fixed for a single allele, while in Rhineland-Palatinate, deviant haplotypes occurred only in two specimens.

The π values of the Rhineland-Palatinate samples for ctr ($\pi=0.04–0.05$ %) are comparable to populations from Baden-Württemberg ($\pi=0.03$ %), Flanders and Alsace (both $\pi=0.06$ %; Neumann et al. 2004), and relict populations in eastern Poland ($\pi=0.02–0.03$ %; Banaszek et al. 2012), but far below East German samples ($\pi=0.3–0.7$ %; Neumann et al. 2004). Likewise, genetic diversity (H) values around 0.1 resemble the other West populations from Alsace, Baden Württemberg and Flanders ($H=0.19–0.2$). The same CCdl01 ctr-haplotype dominates in all West populations, from Belgium to Alsace, while it is only moderately frequent at 0.1–0.55 in most of Central (Neumann et al. 2004). CCdl01 is completely absent in Lower Saxony, where a different founder effect seems to have led to a fixation on CCdl04.

Diversity in Austrian samples ($H=0.7$; $\pi=0.3$ %) is comparable to Moravia ($H=0.8$; $\pi=0.4$ %; Neumann et al. 2004), which also belongs to the Pannonia lineage, even though only three Austrian specimens were sampled. A higher diversity for Austria might be expected. No previous data on COI haplotypes in common hamsters is available, but the Central, Pannonia and Eastern lineages can be expected to be more diverse than the BNN and Upper Rhine populations.

Fixation of alleles is typical for populations of small size, as genetic drift is a more important factor in declining populations. It is also amplified by the formerly occurring considerable population size fluctuations. Random haplotypes become fixed due to increased inbreeding and reduced influx from neighbouring populations. However, in different populations, entirely different haplotypes may become fixed. This is demonstrated by the Rommerskirchen and Zülpich relict populations of North Rhine-Westphalia. Even though both groups live in the Cologne Lowland and cannot be distinguished morphologically, they are nowadays completely isolated at small population sizes below 200 individuals. In both populations, only a single ctr and COI haplotype occurs, but the COI haplotype of the Zülpich population differs from Rommerskirchen in a single character (Fig. 5). Since the Rommerskirchen group's allele, CcCOI01, is also the most common haplotype in Rhineland-Palatinate, it is more likely to be the ancestral state. The new allele could, thus, be a novel mutation in the Zülpich population that was fixed only recently. Differences between the Zülpich and Rommerskirchen (Neuss) populations have also been shown
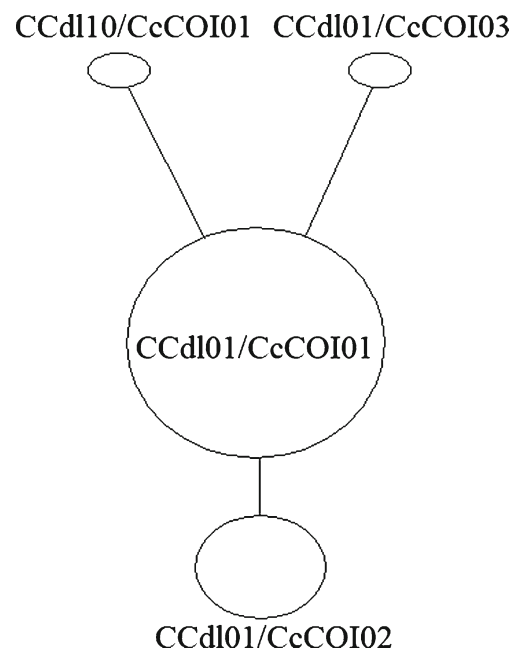
Fig. 5 Statistical parsimony haplotype network of the four combined ctr + COI haplotypes found in West populations of Germany (compare Table 2). *Circle sizes* correlate with haplotype frequency ($n=35$). All changes are transitions

for microsatellite data by La Haye et al. (2011). In their study, the Rommerskirchen population clustered with Dutch and Belgian populations rather than Zülpich. When only specimens of the BNN phylogeographic lineage were included in the statistics, both relict populations formed their own clusters that were distinct from Dutch or Belgian samples. Unfortunately, our sample from the Dutch border (Tüddern) did not yield a COI sequence to compare.

Close ties of the West hamsters to populations of the Thuringian Basin are not only supported by geographical proximity and morphology. CCdl10, the only other ctr haplotype next to CCdl01 recovered in Rhineland-Palatinate, was previously described from Thuringia (Neumann et al. 2004). Additionally, in median-joining networks based on combined ctr and 16S mitochondrial genes, West hamsters are found closest to haplotypes from Thuringia (Neumann et al. 2005). The COI haplotype CcCOI03, found in Rhineland-Palatinate, is also closer to the Austrian outgroup COI haplotypes than any other West haplotype. This is, in all likelihood, an independent homoplasy, but it is possible that this haplotype constitutes a link between West and more eastern populations. To confirm this hypothesis, COI data from Central populations, especially Thuringia is needed. If true, it would further fortify the hypothesis that the northern Upper Rhine area is the origin of West expansion.

The data collected in this study reinforces the status of the westernmost common hamster populations as a clearly distinct phylogeographical lineage, which is not only distinguished from the Central lineage by lower genetic diversity and certain haplotypes but also by quantifiable morphometric fur traits. Differences in the frequency and extent of these traits also allow for the differentiation of two more sublineages within this western complex, the BNN sub-lineage along the Lower Rhine and the Upper Rhine sub-lineage in the Upper Rhine Valley, the two of which are geographically separated by the Rhenish Massif.

Although the West lineage may fulfil the criteria for a subspecies according to Mayr (1963), we do not advocate its formal recognition. This lineage is completely isolated and morphologically distinct, but the degree of genetic differentiation between West and Central is low. The BNN and Upper Rhine populations inhabited these areas in relative isolation for several thousand years and may possess yet unnoticed special adaptations to their habitat. A protection of the remaining autochthonous populations is, thus, essential for conservation and reintroduction efforts.

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