Untersuchungen zur Systematik der Hamster (Cricetinae) sowie zur genetischen Populationsstruktur und Phylogeografie des Feldhamsters *Cricetus cricetus* (Linneaus, 1758) und des Goldhamsters *Mesocricetus auratus* (Waterhouse, 1839)

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1. Einleitung

1.1. Schwerpunkte der Habilitationsschrift

Das Kernstück der vorliegenden Habilitationsschrift bilden Untersuchungen zu zwei Forschungsschwerpunkten. Der erste beschäftigt sich mit der Erstellung einer Systematik für die Unterfamilie der Hamster (Cricetinae) basierend auf molekulargenetischen Daten. Der zweite Schwerpunkt beinhaltet Arbeiten zur genetischen Diversität, genetischen Struktur und Phylogeografie zweier Hamsterarten: dem Feldhamster *Cricetus cricetus* und dem Goldhamster *Mesocricetus auratus.* Darüber hinaus wird die Populationsstruktur des ostmediterranen Türkischen Hamsters *Mesocricetus brandti* im Vergleich zu *M. auratus* analysiert. Kapitel 2, 3 und 4 geben die wesentlichen Ergebnisse der Schwerpunktpublikationen wieder.

Die Liste der habilitationsrelevanten Publikationen enthält weitere Artikel zum Goldhamster *Mesocricetus auratus* und Rattenhamster *Tscherskia triton*, die im Zusammenhang mit der hier dargelegten Problematik von genetischer Diversität und Populationsstruktur der Hamster stehen. Diese Arbeiten werden aber nicht ausführlich diskutiert, da sie nicht vom Habilitanten selbst oder nur anteilig konzipiert wurden. Die entsprechenden Publikationen sind im Text kursiv dargestellt, um sich von den Schwerpunktarbeiten zu unterscheiden.

Des Weiteren erforderten die folgenden genetischen Studien die Entwicklung polymorpher Mikrosatellitensysteme, da bisher keine entsprechenden Marker für Hamster zur Verfügung standen. Dazu mussten eine Reihe molekularer Techniken (z.B. nichtradioaktive Hybridisierungsverfahren, “enrichment“-Techniken) etabliert werden. Im Rahmen dieser Entwicklungen wurden auch Mikrosatelliten für andere Tierarten isoliert. Diese Arbeiten besitzen zwar keinen direkten Bezug auf die genannten Forschungsschwerpunkte, waren aber für die Routineanwendung einer grundlegenden molekularen Methode essentiell und wurden vom Autor durchgeführt. Deshalb werden diese Arbeiten in der folgenden Schrift nicht kommentiert, sind aber in der Liste der habilitationsrelevanten Publikationen aufgeführt.

1.2. Zur Biologie der Unterfamilie der Hamster (Cricetinae, Rodentia, Mammalia)

Die echten Hamster (Cricetinae) bilden eine Unterfamilie der Nagetiere (Rodentia), deren Verbreitung auf die Paläarktis beschränkt ist. Merkmale sind u. a. brachydonte, mit zwei Längsreihen von Tuberkeln versehene Molare. Die Molaren M_2 und M_3 besitzen meist keinen oder nur einen reduzierten Mesoloph. Der erste und zweite obere Molar besitzt vier Wurzeln und der erste Höcker des M^1 ist in zwei Teile gespalten. Die Cricetinae haben charakteristische, interne Backentaschen, wobei der *Musculus buccinator*, ein Derivat des Trapeziusmuskels, als Rückzieher fungiert. Hamster sind mäuse- bis rattengroß mit kurzen breiten Füßen und kurzen Schwänzen (Ausnahme: Rattenhamster *Tscherskia triton*). Ihr kurzer dichter Pelz wurde mitunter kommerziell genutzt, wie im Falle des Feldhamsters *Cricetus cricetus*.

Hamster leben subterran und sind vor allem dämmerungs- und nachtaktive Nager. Sie gelten als vorwiegend solitär. Eine ausgeprägte soziale Toleranz und sogar biparentales Verhalten findet sich nur bei der Gattung *Phodopus* (Wynne-Edwards und Lisk 1987; Wynne-Edwards 1995, 2003). Die Vertreter der Cricetinae bewohnen offene Landschaften, meist halbtrockene oder trockene Steppenbiotope, in denen sie mehr oder weniger ausgedehnte Bausysteme anlegen. Einige Arten leben auf klimatisch günstig gelegenen Gebirgsplateaus, wie Raddes Hamster *Mesocricetus raddei*. Der Rattenhamster *Tscherskia triton* ist die einzige Hamsterart, die auch auf feuchteren Wiesenstandorten angetroffen wird. Dagegen bevorzugt der Roborovski-Zwerghamster *Phodopus roborovskii* sehr trockene Biotope und dringt in wüstenartige Dünenfelder vor. Mit der Besiedlung von Extremhabitaten könnte der kleinste Vertreter der Cricetinae z.B. dem Konkurrenzdruck anderer Hamsterarten ausweichen. Die meisten der rezenten Hamsterspezies findet man in den kontinentalen Steppen Zentralasiens, in China und der Mongolei, welche wahrscheinlich das evolutionäre Zentrum für die meisten europäischen und asiatischen Hamsterarten bildeten. Lediglich die Gattung *Mesocricetus* hat ihren Ursprung im ostmediterranen Raum (De Bruijn et. al. 1970; Vasileiadou et al. 2003; Storch 2004). Als Offenlandarten kommen Hamster häufig auf landwirtschaftlich genutzten Flächen vor (Niethammer 1982; Nechay 2000; Gattermann et al. 2001; Xie und Zhang 2005). Ein gutes Nahrungsangebot, geeignete mikroklimatische Verhältnisse, tiefe Böden und ausreichende Deckungsmöglichkeiten führen hier häufig zu ungewöhnlich hohen Populationsdichten (Calinescu 1931; Stubbe et al. 1998; Zhang und Wang 1998). Deshalb wurden und

werden Hamster in weiten Teilen Europas und Asiens auf Agrarflächen bekämpft. In Europa kommen 5 Arten vor; Feldhamster *Cricetus cricetus*, Eversmann-Hamster *Allocricetulus eversmanni*, Grauer Hamster *Cricetulus migratorius*, Rumänischer Hamster *Mesocricetus newtoni* und Raddes Hamster *Mesocricetus raddei*, davon besitzen *C. cricetus* und *M. newtoni* einen internationalen Schutzstatus (Panteleyev 1998; Mitchell-Jones et al. 1999).

Obwohl Hamster im eurasischen Raum weit verbreitet sind, existierten bisher kaum Untersuchungen zur genetischen Struktur einzelner Arten. Dies steht im Gegensatz zur großen Anzahl von Studien über andere Muriden-Gattungen z.B. *Microtus* (Jaarola und Searle 2002; Brunhoff et al. 2003; Haynes et al. 2003; Galbreath und Cook 2004)*, Clethrionomys* (Tegelström et al. 1988; Gerlach und Musolf 2000; Deffontaine et al. 2005) oder *Apodemus* (Michaux et al. 2003, 2004). Erst 2003 und 2005 entstanden, neben den hier dargelegten Arbeiten, zwei weitere Publikationen zum Feldhamster und Rattenhamster (Smulders et al. 2003; Xie und Zhang 2005). Ähnlich verhält es sich mit der Anwendung molekularer Marker für die Systematik der Unterfamilie Cricetinae. Bisher gab es nur Ansätze für DNA-basierende Taxonomien (Potapov et al. 1994; Michaux et al. 2001; Lebedev et al. 2003; Steppan et al. 2004). Zwei Standardwerke der Säugetiersystematik, Corbet (1978) und Wilson und Reeder (1993) wiesen daraufhin, dass eine Überarbeitung der Cricetinae-Taxonomie dringend erforderlich wäre. Einige Hamsterarten dienen als wichtige Modelltiere in der biologischen und medizinischen Forschung. Der Goldhamster *Mesocricetus auratus* ist ein weit verbreitetes Labortier und wird insbesondere zur Aufklärung der Mechanismen biologischer Rhythmen genutzt. Die Gattung *Phodopus* zeigt eine inter- und wahrscheinlich auch intraspezifische Variabilität bei der biparentalen Brutpflege (Neumann et al. eingereicht), welches sie zu einem idealen System zur Erforschung der Evolution sozialen Verhaltens bei Säugern macht. Kenntnisse zur genetischen Diversität, Populationsstruktur und Systematik wildlebender Hamster bilden daher eine wesentliche Grundlage, um Laborbefunde zu überprüfen. Darüber hinaus besteht die Möglichkeit, einzelne Phänomene (z.B. Verhaltensäußerungen) phylogenetischen Linien zuzuordnen, um die Umstände (genetische Voraussetzungen, Umweltparameter) für deren Herausbildung besser zu analysieren.

2. Phylogenie und Systematik der Cricetinae

Trotz einer Reihe morphologischer (Argyropulo 1933; Vorontsov 1960; Hamar und Schutowa 1966), zytologischer (Matthey 1960; Radjabli 1975; Gamperl et al. 1978) und karyologischer Studien (Kartavtsev et al. 1984a, 1984b) ist die Systematik der Hamster und die phylogenetische Verwandtschaft rezenter Hamstergattungen stark umstritten. Weitverbreitete Systematiken wie Wilson und Reeder (1993) führen sieben Gattungen: *Allocricetulus*, *Cansumys*, *Cricetulus*, *Cricetus*, *Mesocricetus*, *Phodopus* und *Tscherskia* mit insgesamt 18 Arten auf. Dagegen beschreibt Corbet (1978) nur vier Gattungen mit 14 Arten (ohne *Calomyscus*). Die häufig als echte Cricetinae geführten Maushamster *Calomyscus* (Flint 1966; Corbet 1978; Pantelejev 1998) vertreten aufgrund morphologischer, zytologischer und genetischer Unterschiede eine andere evolutionäre Linie (Matthey 1960; Michaux et al. 2001; Steppan et al. 2004).

Es wird angenommen, dass sich die Unterfamilie Cricetinae von den Democricetodontini ableitet, einer Nagergruppe, die im frühen und mittleren Miozän in der nördlichen Hemissphäre weit verbreitet war (Fahlbusch 1969; Chaline 1977; Chaline und Mein 1979). Die fossile Hamstergattung *Cricetulodon* könnte sich z.B. aus einem *Democricetodon*-Zweig entwickelt haben und eine evolutive Brücke zur Cricetinae-Gattung *Rotundomys* bilden (Freudenthal et al. 1998). Bisher wurde eine größere Anzahl fossiler Hamstergattungen beschrieben. Carleton und Musser (1984) nennt acht ausgestorbene Gattungen: *Cricetulodon* (seit dem mittleren Miozän), *Kowalskia* (seit dem späten Miozän), *Rotundomys* (seit dem späten Miozän), *Collimus* (frühes Pliozän), *Microtocricetus* (frühes Pliozän), *Sinocricetus* (frühes Pliozän), *Nannocricetus* (frühes Pliozän), *Rhinocricetus* (frühes und Mittel-Pleistozän). McKenna und Bell (1997) listen noch acht weitere Gattungen, wovon fünf bereits aus dem späten Miozän stammen. Viele fossile Hamstertaxa haben ihren Ursprung im Miozän. Die Ursache dafür liegt in einem globalen Faunenwandel, der sich in dieser Zeit vollzog (Webb und Opdyke 1995; Janis et al. 2000). Tektonische Veränderungen und ein zunehmend trockneres Klima führten zur Formation weiter, offener Landschaften, die Steppenelementen bessere Lebensbedingungen boten (Cerling et al. 1997; Agustí et al. 1999; Fortelius et al. 2002). Alle rezenten Hamstergattungen sind durch Fossilien belegt. Nachweise von *Cricetus*-Hamstern aus dem Miozän und weiten Teilen des Pliozäns sind aber wahrscheinlich einer ausgestorbenen Gattung *Pseudocricetus* (Topachevski und Skorik 1992) oder *Apocricetus* (Freudenthal et al. 1998) zuzuord-

nen und stimmen nicht mit der rezenten Gattung *Cricetus* überein. Äußerst schwierig dagegen gestaltet sich die phylogenetische Ableitung heutiger Hamstergattungen von fossilen Taxa (Abb. 1). Einige Autoren betrachten die Gattung *Kowalskia* als Vorläufer von *Tscherskia*, *Cricetus* und *Allocricetulus* (De Bruin 1976; Gromov und Baranova 1981; Topachevski und Skorik, 1992), wogegen nach Fahlbusch (1969) *Cricetus* und *Cricetulus* direkt von *Democricetodon* abstammen. Interessanterweise hat man bisher keine fossile Hamstergattung gefunden, die als Ausgangspunkt der rezenten Gattung *Mesocricetus* infrage käme.

Die Publikation **„Molecular phylogeny of the Cricetinae subfamily based on the mitochondrial cytochrome *b* and 12 SrRNA genes and the nuclear vWF gene“** ist der erste umfangreichere Versuch bestehende systematische Unklarheiten mit Hilfe eines genetischen Ansatzes zu klären und eine Phylogenie der Unterfamilie Cricetinae unter Berücksichtigung von Fossilienfunden und paläoklimatischen Daten zu erstellen. Mit der Anwendung molekularer Uhren wurde ein Zeitrahmen für die Evolution wichtiger Hamsterlinien, sowie deren weitere Aufspaltung berechnet. Bei den anhand von genetischen Distanzen errechneten Zeiträumen wurde den zunehmenden Befunden zur Heterogenität von Substitutionsraten innerhalb und zwischen verschiedenen DNA-Abschnitten Rechnung getragen (Howell et al. 2004; Ho et al. 2005). Die Zeitschätzungen erfolgten unter den Annahmen einer konstanten Uhr und einer “relaxed clock“. Die genetischen Stammbäume wurden auf der Basis von Sequenzinformationen dreier partieller Gene erstellt. Es handelt sich dabei um zwei mitochondriale Gene (Cytochrom b, 12SrRNA) und einen nukleären Abschnitt (von Willebrand-Faktor, Exon 28). Phylogenetische Stammbäume wurden mit Hilfe von Maximum-Likelihood, Maximum Parsimony, Distanz- und Bayesischen Methoden konstruiert. Ein wesentliches Ergebnis der Arbeit ist die Existenz von drei phylogenetischen Hauptlinien innerhalb der rezenten Vertreter der Cricetinae: *Phodopus*-Gruppe, *Mesocricetus*-Gruppe, *Cricetus*-Gruppe (*Cricetus*, *Cricetulus*, *Allocricetulus*, *Tscherskia*). Alle drei Linien entstanden im oberen bis späten Miozän, was gut mit der großen Anzahl heute ausgestorbener fossiler Gattungen aus dieser Zeit übereinstimmt. Das hohe evolutionäre Alter der *Phodopus*-Linie überrascht, da Fossilien der Gattung erst aus dem Pleistozän bekannt sind (Fahlbusch 1969; Shaohua 1984; Shaohua und Cai 1991). Die molekularen Daten zeigen dagegen, dass eine Aufspaltung der *Phodopus*-Hamster bereits am Ende des Miozäns oder im Übergang vom Miozän zum Pliozän stattfand.

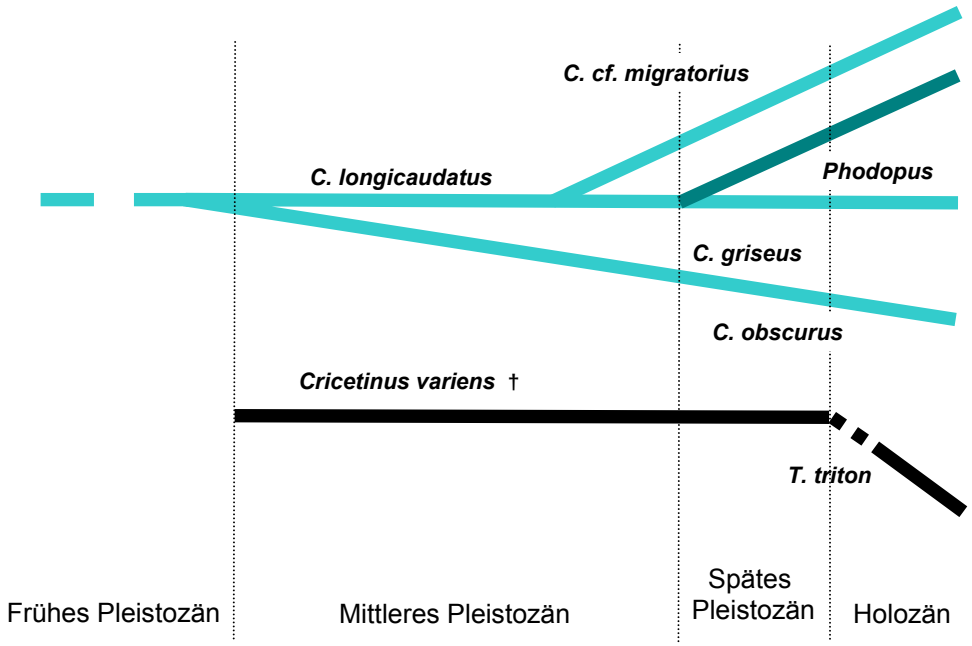

Abb. 1 Phylogenie der bei Zhoukoudian / China nachgewiesenen fossilen Cricetinae basierend auf ihrem biochronologischen Auftreten in den verschiedenen Fundschichten und morphologischen Merkmalen (verändert nach Shaohua 1988). Abkürzungen: *C = Cricetulus*, *T = Tscherskia.*

Schwierigkeiten bei der Identifizierung von fossilen Überresten, insbesondere kleiner Hamstergattungen (*Phodopus* und *Cricetulus*), könnten dafür eine Erklärung liefern (Schaub 1930; Shaohua und Cai 1991; Kowalski 2000). Genetische Stammbäume identifizieren die Gattung *Mesocricetus* als eine separate phylogenetische Linie. Dies widerspricht der Annahme einer engen Verwandtschaft mit *Cricetus* (Argyropulo 1933) und unterstützt Niethammer (1982), der auf morphologische Sondermerkmale der *Mesocricetus*, wie z.B. größere Zitzenzahl und anders gestaltete Anteorbitalplatten hinwies. Eine wichtige neue Erkenntnis ist die fehlende Monophylie der Gattung *Cricetulus*. Der graue Zwerghamster *Cricetulus migratorius* steht den Gattungen *Tscherskia*, *Cricetus* und *Allocricetulus* deutlich näher, als allen untersuchten Vertretern der Gattung *Cricetulus.* Ein Befund, der ebenfalls gängigen Literaturmeinungen widerspricht, ist die nahe genetische Verwandtschaft von *Mesocricetus auratus* und *M. raddei.* Nach Auswertung karyologischer und morphologischer Merkmale postulierten Hamar und Schutowa (1966) die Existenz einer transkaukasischen (*M. auraus, M. brandti, M. newtoni*) und einer ciskaukasischen Linie (*M. raddei*) innerhalb der Gattung *Mesocricetus*. Dies konnte anhand der DNA-Vergleiche nicht bestätigt werden. Die enge Assoziation von *M. auratus* und *M. raddei* könnte auf einen Ursprung des Goldhamsters im südlichen Vorland des Kaukasus deuten. Eine Entstehung der Art in Palästina erscheint eher unwahrscheinlich (Hosey 1982). Im Rahmen

der Publikation wird auch versucht, eine biogeografische Erklärung für die longitudinale Verbreitung einer Reihe von sehr nah verwandten Hamsterarten (*Allocricetulus eversmanni* - *A. curtatus*, *Phodopus campbelli* - *P. sungorus*, *C. barabensis* - Gruppe) in Zentralasien zu finden. Diese Arten entstanden wahrscheinlich im Laufe des Pleistozäns durch Expansion aufgrund der sich nach Westen ausdehnenden Steppen. Dabei scheinen die Gebirgsausläufer des Sajan und Altai als wichtige geografische Barrieren zu fungieren. Leider existieren keine genetischen Untersuchungen zu anderen Steppensäugern dieser Region, um diese Hypothese weiter zu überprüfen.
Mit der vorgelegten Phylogenie konnte erstmals eine Zuordnung rezenter Hamstergattungen zu einzelnen evolutionären Entwicklungslinien erfolgen. Bisher waren die verwandtschaftlichen Beziehungen der meisten beschriebenen Gattungen unklar (Carleton und Musser 1984; Niethammer 1982). Die vorgelegte molekulare Phylogenie ermöglicht auch, die große Anzahl fossiler Hamstergattungen in eine exaktere zeitliche und verwandtschaftliche Beziehung mit rezenten Arten zu setzen. Die teilweise Diskrepanz zwischen molekularen Zeitschätzungen und Fossilien-Datierungen machen eine Überprüfung von paläontologischen Funden erforderlich. Allerdings besitzen auch molekulare Schätzungen durch variierende Substitutionsraten und “lineage sorting“ eine gewisse Unschärfe (Ingman et al. 2000; Lister 2004; Howell et al. 2004; Ho et al. 2005). Obwohl eine Reihe systematischer Unklarheiten innerhalb der Cricetinae, so z.B. die Validität der Gattungen *Tscherskia* und *Allocricetulus*, geklärt werden konnten, verbleibt ein hoher Forschungsbedarf. Wichtiges Augenmerk ist dabei auf die Gattung *Cricetulus* zu richten, die offensichtlich nur ein Sammelbecken morphologisch ähnlicher Hamster darstellt.

Seit 2004 läuft ein Forschungsprojekt zur molekularen Systematik der Gattung *Cricetulus* in Zusammenarbeit mit Dr. V. Lebedev (Zoologisches Museum der Staatlichen Universität Moskau) und Dr. A. Surov (Severtzov Institut für Tierökologie und Evolution, RAS, Moskau). In diesem Projekt wird versucht, ein Diagnosesystem mit Hilfe genetischer und morphologischer Charakeristika für die Identifizierung von *Cricetulus*-Arten/Unterarten zu erstellen.

2.1.1. Molecular phylogeny of the Cricetinae subfamily based on the mitochondrial cytochrome b and 12S rRNA genes and the nuclear vWF gene

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ABSTRACT

Despite some popularity of hamsters as pets and laboratory animals there is no reliable phylogeny of the subfamily Cricetinae available so far. Contradicting views exist not only about the actual number of species but also concerning the validity of several genera. We used partial DNA sequences of two mitochondrial (cytochrome *b*, 12SrRNA) and one partial nuclear gene (von Willebrand Factor exon 28) to provide a first gene tree of the Cricetinae based on 15 taxa comprising six genera. According to our data, Palaearctic hamsters fall into three distinct phylogenetic groups: *Phodopus*, *Mesocricetus* and *Cricetus*-related species which evolved during the late Miocene about 7 – 12 MY ago. Surprisingly, the genus *Phodopus*, which was previously thought to have appeared during the Pleistocene, forms the oldest clade. The largest number of extant hamster genera is found in a group of *Cricetus*-related hamsters.

Das Projekt zur Systematik der Cricetinae wurde von mir konzipiert. Die genetischen Analysen des Cytochrom B-Gens und von Willebrand Faktors wurden durch mich unter der technischen Assistenz der Laborantinnen Frau Becke und Frau Gebhardt im eigenen Labor durchgeführt. Die DNA-Sequenzen für das 12SrRNA-Gen wurden von V. Lebedev in Moskau bereitgestellt. Das Sammeln der Hamsterproben erfolgte durch eigene Exkursionstätigkeit und durch die Mitautoren. Die statistische Auswertung erfolgte durch J. Michaux, V. Lebedev und mich. Das Manuskript wurde durch mich erstellt. Der Material und Methodenteil wurde von Dr. J. Michaux, V. Lebedev und mir erarbeitet. Dr. A. Surov führte die Revision der Verbreitungskarten durch.

The genus *Cricetulus* itself proved to be not truly monophyletic with *Cricetulus migratorius* appearing more closely related to *Tscherskia*, *Cricetus* and *Allocricetulus*. We propose to place the species within a new monotypic genus. Molecular clock calculations are not always in line with the dating of fossil records. DNA based divergence time estimates as well as taxonomic relationships demand a reevaluation of morphological characters previously used to identify fossils and extant hamsters.

INTRODUCTION

The subfamily Cricetinae comprises a group of mouse- to rat-sized rodents with characteristic cheek pouches and short tails. Important dental characteristics distinguishing extant Cricetinae are brachydont cuspidate molars with conspicuously bifurcated anterocones, an absent or reduced mesoloph and upper M1, M2 with four roots. Most hamster genera are described from the late Miocene and peaks in species diversity seem to coincide with cooler periods during the following Plio- and Pleistocene (Argyropulo, 1938; McKenna and Bell, 1997; Kowalski, 2001). Hamsters are semi-fossorial and live mainly solitary. They inhabit steppe and semi-arid habitats as well as agricultural land throughout the Palaearctic. Some hamster species show occasional gradations and are persecuted as pests in crop lands. Two species; the golden hamster *Mesocricetus auratus* and the Chinese hamster *Cricetulus griseus* became important laboratory animals for biological and medical research.

The origin of Cricetinae is often associated with Democricetodontini (sometimes included in Megacricetodontini) a widely distributed tribe in the northern hemisphere during the early and middle Miocene (Fahlbusch, 1969). The relationships of Cricetinae with some other Neogene lineages which share characters of voles and hamsters such as Microtocricetinae and Ischymomyinae remain unclear. Genetic studies of hamster taxonomy are mainly restricted to cytological (e.g. Matthey, 1960; Rajabli, 1975; Gamperl et al., 1978) and allozyme studies (Kartavtsev et al., 1984a; 1984b). There is a fair amount of morphological data about the group (e.g. Vorontsov, 1982) but they do not provide enough reliable synapomorphies for phylogenetic inference. In this respect, the reconstruction of ancestral lines of extant hamster species on the basis of fossils proves complicated and often questionable (Hir, 1997; Kowalski, 2001). The current systematics of the subfamily is confusing and the actual number of species as well as the phylogenetic relationship between genera is highly disputed (e.g. Corbet, 1978; Wilson and Reeder, 1993). Mouse-like hamsters of the genus

Calomyscus are occasionally considered as Cricetinae (Corbet, 1978; Pantelejev, 1999) but morphological, cytological (Matthey, 1961) and genetic data (Michaux et al., 2001; Steppan et al., 2004) disprove such a view. For that reason we excluded *Calomyscus* from analyses.

Our study aimed to provide a first genetically based systematic framework for the Cricetinae examining a representative number of species. Furthermore, we evaluated the controversial position of taxa like *Allocricetul*us and *Tscherskia* and attempted to resolve the phylogenetic relationship of extant hamster genera.

MATERIAL AND METHODS

Animal material

This study includes samples of 15 hamsters mainly collected in their natural distribution area (Table 1). Only *Cricetulus griseus, Phodopus roborovskii*, *Phodopus sungorus* and *Phodopus campbelli* were obtained from laboratory strains kept at the Institute of Zoology, Martin-Luther-University Halle-Wittenberg, Germany and Severtzev Institute of Animal Ecology and Evolution in Moscow, Russia. Distribution ranges of the analysed species are described in Figure 1. DNA data were usually obtained from the same individual except for a few species where genes had been sequenced from two individuals belonging to different populations. Such animals and genes are marked in table 1.

DNA sequencing of cytochrome b, 12 S rRNA and von Willebrand Factor exon 28

DNA was extracted from ethanol preserved or fresh tissues following a standard protocol supplied with the E.Z.N.A. Tissue DNA Kit II system (peqlab Biotechnologie). Gene amplification was carried out using the Ready-To-Go-system (Amersham). Forty pmol of each primer and 0.2 µg of genomic DNA were added to a total volume of 50 µl. PCR was run in a thermocycler UNO II, Biometra employing 35 cycles (60 s at 94°C, 60 s at primer specific annealing temperature and 90 s at 72°C) with an inital heating step of 4 min at 94°C and a final extension cycle of 10 min at 72°C.

A large portion of cytochrome *b* (cyt*b*) was amplified and sequenced as described in Neumann et al. (2005).

PCR and sequencing of the near complete 12SrRNA (12S) was achieved according to the procedure given in Kuznetsov et al., (2001) using the following PCR primers: Phe389L: 5'-GGCACTGAAAATGCCTAGATG-3', 1379H: 5'-CCAAGCACACTTTC-CAGTATG-3', 842L: 5'-CAAACTGGGATTAGATACC-3', 1015: H: 5'-GGT [G/A] AGGT[T/C]TATCGGGGTTT-3', 860H: 5'-GGTATCTAATCCCAGTTTG-3' and L329: 5'-AAAGCAAAGCACTGAAAATG-3'/ H618: 5'-TATCGATTATAGAACAGGCTCC-3'. Amplification and sequencing of von Willebrand Factor (vWF) exon 28 followed Huchon et al. (1999).

Table 1 Hamster species used for DNA sequencing and their origins. Question marks indicated genera which are not supported by DNA data. (*) marks species with sequences obtained from animals of two different populations.

Species group	Genus	Species	Locality	Tissue
	Phodopus	*P. roborovskii*	Lab strain	liver
	Phodopus (?)	*P. campbelli*	Lab strain	liver
		P. sungorus	Lab strain	liver
	Cricetulus	*C. longicaudatus*	Buriati/Russia	liver
"Dwarf		*C. barabensis**	Uur Gol/Mongolia; N.Khangai/Mongolia	liver
Hamster"		*C. griseus*	Lab strain	liver
	Cricetulus (?)	*C. migratorius**	Kayseri, Central Anatolia/Turkey; Volgograd/Russia	liver
	Allocricetulus	*A. eversmanni*	Saratov region/Russia	liver
	Tscherskia	*T. triton**	near Beijing/China; Ussuriisk reg./Russia	liver
	Mesocricetus	*M. raddei nigriculus*	Mozdok, Ossetia/Russia	liver
"Middle		*M. raddei avaricus*	Dagestan, Kunzakh/Russia	liver
Hamster"		*M. auratus*	Aleppo/Syria	liver
		M. brandti	Central Anatolia/Turkey	liver
		M. newtoni	Shumen/Bulgaria	liver
"Large Hamster"	*Cricetus*	*Cr. cricetus**	Hakel, Saxony-Anhalt/Germany Mozdok, Ossetia/Russia	liver

Sequence alignment and saturation analysis

Partial genes were compared to published genes (GenBank) to confirm gene identity. New sequences were aligned with the ED editor (MUST package, (Philippe, 1993). The program AFAS (MUST package) was used to combine the aligned matrices of cyt*b*, 12S and vWF.

All three genes were examined for saturation following Philippe and Douzery (1994) and Hassanin *et al.* (1998). Using the matrices of patristic and adjusted character distances calculated by PAUP* 4.0b8 (Swofford, 1998), the pairwise numbers of observed differences were plotted against the corresponding values for inferred substitutions. The slope of the linear regression (S) was used to evaluate the level of saturation. When no saturation is observed in the data set, the slope equals one whereas the slope tends towards zero as the level of saturation increases.

Phylogenetic reconstructions

The level of incongruence between genes was tested using PAUP*4.0b8 (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).

As the ILD test showed a high congruence between the three studied genes (see below for details), phylogenetic analyses were performed using the combined matrix for cyt*b* (924 bp), 12S (973 bp) and vWF (789 bp) sequences.

The aligned sequences were analysed by distance (neighbour joining, NJ; Saitou and Nei, 1987), maximum parsimony (MP) (Fitch, 1971) and maximum likelihood (ML) methods using PAUP* 4.0b8. Model test version 3.06 (Posada and Crandall, 1998) was used to determine the best-fit substitution model for the Cricetinae data. According the results of this test (using the AIC criteria), the General Time Reversible (GTR) model was used for the distances and ML analyses. To take into account differences of substitution rates across sites, the GTR analysis was performed assuming a gamma distribution at eight categories. The alpha parameter (Yang, 1996) and the proportion of invariant sites (I) were estimated with the ML method in PAUP* 4.0b8. MP analyses were performed using heuristic search and TBR branch swapping option. The robustness of inferences was assessed by bootstrapping (BP) (1000 random repetitions for MP and distance analyses, and 100 for ML). A Bayesian approach to phylogeny reconstruction (Yang and Rannala, 1997; Huelsenbeck et al., 2001) was also used, implemented in MrBayes 3.1.1. (Huelsenbeck and Ronquist, 2001). Metropolis-coupled Markov chain Monte Carlo sampling was performed with four chains that were ran for 3 millions generations, using default model parameters as starting values. Bayesian posterior probabilities were picked from the 50% majority rules consensus of trees sampled every 100 generations, after removing trees

obtained before chains reached apparent stationarity ("burn in" determined by empirical checking of likelihood values). This analysis was repeated 10 times to check if the chains always converged on a similar distribution and if the corresponding results were the same. Finally, the analysis was performed with separate models for each gene. Each model of evolution was estimated using MrBayes 3.1.1.

Molecular clocks and divergence time

In order to determine divergence times within *Cricetinae,* we used two approaches. At first we applied a global clock to a linearized tree as described in Michaux et al. (2001). Secondly, we imposed a relaxed clock which allows for a heterogenous substitution rate according to Thorne et al. (1998). Both methods already produced overlapping results (Michaux et al, 2001; Steppan et al. 2004). To apply a general clock we checked the assumption of rate constancy with the help of both relative-rate and likelihood-ratio tests. To test for rate differences of cyt*b*, 12S and vWF between the different Cricetinae genera, relative-rate tests were conducted with each of them against the remaining lineages. The relative-rate tests were done with RRTree, version 1.0 (Robinson *et al.*, 1998) which improves the test of Wu and Li (1985) by taking into account taxonomic sampling and phylogenetic relationships. The three DNA regions were analyzed separately. The ML tree for each gene was chosen as the reference topology. According to the muroid phylogeny obtained by Michaux et al. (2001) and Steppan et al. (2004), *Cricetomys gambiana* and *Nesomys rufus* were used as outgroups. For coding sequences (cyt*b*, vWF), relative-rate tests were performed on the proportions of synonymous (Ks) and nonsynonymous (Ka) substitutions. For non coding regions (12S), relative-rate tests were performed on the proportion of all the substitutions types (K). Likelihood-ratio tests were performed as proposed by Felsenstein (1981) for each gene separately and calculated in PAUP*4.0b8 using the ML tree for the combined data as the reference topology within Cricetinae. The outgroup comprised *Cricetomys*, *Nesomys*, *Mus*, *Rattus*, *Tatera*, *Gerbillus*, *Clethrionomys* and *Peromyscus* (just the latter two for 12S); cladistic relationships between them were accepted as given in Steppan et al. (2004).

We also estimated divergence times using the relaxed Bayesian clock method for multigene data (multidivtime program – Thorne and Kishino, 2002). The ML topology for the combined data was used as the reference tree. Only closest outgroups (*Peromyscus* and *Clethrionomys*) were included in the 12S matrix due to ambiguities

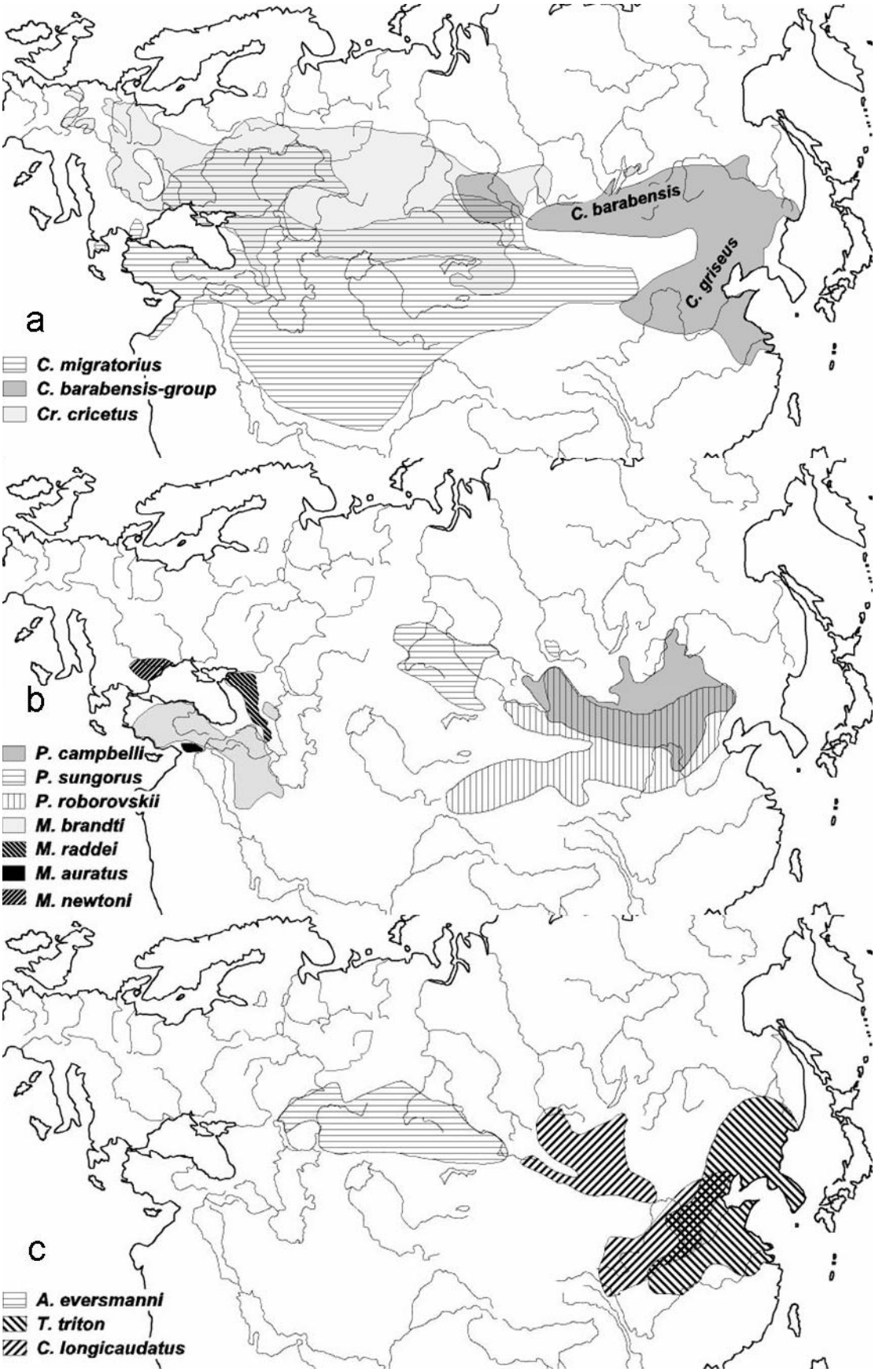

Fig. 1a, b, c Geographical distribution of sampled Cricetinae species. The maps are based on Panteleyev (1999) with revisions. Abbreviations are as follows: *P – Phodopus*, *M – Mesocricetus*, *A – Allocricetulus*, *T – Tscherskia*, *C – Cricetulus*, *Cr – Cricetus.*

in alignments with sequences from distant taxa such as *Cricetomys* and *Tatera*. The F84 +G model was applied independently to each of the within-gene partititions such as codon positions for vWF and cyt*b* or stems and loops for 12S. Model parameters were estimated with the use of baseml software (PAML version 3.14 – Yang, 1997). Branch lengths and their covariance matrices were calculated with estbranche-dna program (Multidistribute package -Thorne and Kishino, 2002). Multidivtime program was run with the Markov chain sampled 10,000 times, every 100th generation. The burn in was set at 10,000 generations. The following prior distributions were accepted: 20 MY (SD = 20 MY) for the expected time between tip and root of the tree corresponding to the split between Murinae+Gerbillinae and Arvicolinae+Cricetinae+Peromyscinae lineages; 0.0075 (SD = 0.040) substitutions per site per million years for the mutational rate at the root of the tree. The priors for rate roughly correspond to the median tip-to-root distance among lineages and partitions divided by 20 MY. Relatively large values for standard deviations (> 4 x mean) were selected to accommodate rate variation among genes and codon positions. The prior for Brownian motion constant was 1.0 (SD = 1.0). Two nodes were subject to time constraints (derived from paleontological data: Jaeger et al., 1986; Jacobs et al., 1989; Jacobs and Downs, 1994; Jacobs et al., 1990): *Mus*/*Rattus* split (12 and 14 MYA), while corresponding upper and lower limits for *Tatera*/*Gerbillus* dichotomy were attributed to 7 and 9 MYA (Tong, 1989).

RESULTS

New sequences

All sequences generated in the present study were deposited in EMBL (cyt*b*: **AJ973378**-**AJ973392**), (12S: **AY997819-25**, **AY997828**, **AY997830**, **AY997832-35**), (vWF: **AM000037-AM000051**).

The alignment of the cyt*b* gene consists of 924 nucleotides for 17 taxa, 387 (42%) of which are variable and 295 (32%) parsimony-informative. The average ratio of TS/TV is 1.56, ranging from 0.98 to 5.87. The complete alignment of the 12S mitochondrial gene comprises 973 sites for 17 individuals. When the indels are removed from the matrix, 846 sites are included in the analyses. Of these 177 (21 %) are variable and 126 (15 %) parsimony-informative. The average ratio of TS/TV is 2.67, ranging from 1.47 to 6.5. The alignment of the vWF sequences of 17 taxa (15 Cricetinae + 2 Arvicolinae as outgroups) comprises 789 nucleotides of which 176 (22 %) are variable

and 94 (12 %) parsimony informative. The average ratio of TS/TV is 3.12, ranging from 1.59 to 7.5**.** The concatenated data matrix for the 17 taxa comprises 2548 nucleotides, 775 (30 %) variable and 537 (21%) parsimony-informative.

Table 2 Degree of within and between genus divergence (K2P distances) for the cyt*b*, 12S and vWF genes.

Taxa compared	**cyt*b***	**12S**	**vWF**
Phodopus/ other Cricetinae (node 1)	22.6	8.6	7.6
Mesocricetus/ Cricetulus, Tscherskia, Cricetus, Allocricetulus (node 2)	20.1	8.3	6.7
C. longicaudatus, C.griseus, C. barabensis/ T. triton, C. migratorius, Cr. cricetus, A. eversmanni (node 3)	20.0	7.6	3.8
P. roborovskii/ P. campbelli, P. sungorus (node 4)	18.4	5.2	4.6
M. brandti, M. newtoni/ M. auratus, M. raddei (node 5)	11.5	4.7	1.1
P. campbelli/ P. sungorus (node 6)	4.5	1	0.5
M. auratus/ M. raddei (*avaricus, nigriculus*) (node 7)	6.3	3.4	1.5
M. raddei avaricus/ M. raddei nigriculus (node 8)	2.3	/	0
M. brandti/ M. newtoni (node 9)	9.8	3.5	1
C. longicaudatus/ C. griseus, C. barabensis (node 10)	15.2	3	1.1
C. griseus/ C. barabensis (node 11)	4.3	0.9	1
T. triton/ C. migratorius, Cr. cricetus, A. eversmanni (node 12)	21.1	7.6	3.5

Saturation analysis

Saturation analysis of the cyt*b* data showed that transitions (TS) and transversions (TV) at positions 1 and 2 and TV at position 3 are moderately affected by homoplasy (TS 1: S = 0.69; TV 1: S = 0.80; TS 2: S = 0.64; TV 2: S = 0.99; TV 3: S = 0.53). On the contrary, transitions at third position, are highly saturated (S = 0.13). Saturation analyses of the 12S data sets were performed on four partitions: transitions and transversions were analyzed separately in loops and stems regions. There is no saturation for TV in stems (S = 0.99) and only moderate saturation for TS in stems (S = 0.75) and for both substitution types in loops (TS: S = 0.70; TV: S = 0.67). Saturation analysis of the vWF data set (17 taxa, 789 nucleotides) indicates that there is no saturation for transitions (TS) and transversions (TV). Therefore, all further analyses were conducted using all events of the three DNA regions, with the exception of TS at third codon position for cyt*b*.

Phylogenic relationships between the different Cricetinae genera

The ILD test showed no significant incongruence between the two mitochondrial genes (p = 0.25) as well as between these ones and vWF (p = 0.17 and 0.56, respectively). Therefore, we concatenated these three genes to enhance the power of resolution. It should be mentioned that we also performed single gene analyses (data not shown) which produced largely similar phylogenetic trees validating the results of the concatenated sequence approach. Some differences inside the *Cricetus*-related clade (cyt*b*/12S) have either low bootstrap support or seem to be random due to the low number of informative sites (position of *M. auratus* in vWF trees). The combined analysis was performed with Arvicolinae (*Clethrionomys glareolus*) and Neotominae (*Peromyscus leucopus*) as outgroups. A consensus tree, constructed from the topologies retrieved by MP, ML, NJ and Bayesian probabilities 5 (BAP) is presented in figure 2. The monophyly of the Cricetinae is strongly supported (MP = 87, NJ = 99, ML = 98, BAP = 1.0). The most basal Cricetine genus is *Phodopus* (MP = 100, NJ = 100, ML = 100, BAP = 1.0). Two main groups can be distinguished for the remaining Cricetinae taxa: a *Mesocricetus*- group and a diversified lineage corresponding to the genera *Cricetulus, Tscherskia, Cricetus* and *Allocricetulus*. The monophyly of both groups is well supported (MP = 100, NJ = 100, ML = 100, BAP = 1.0). Within these three main groups, different subclades are robustly supported:

- *Phodopus campbelli* and *P. sungorus* appear closely related (MP = 100, NJ = 100, ML = 100, BAP = 1.0) and separated to *P. roborovskii*.

- *Mesocricetus auratus* is nested with *M. raddei* (subspecies *avaricus* and *nigriculus*) in a same subclade (MP = 100, NJ = 100, ML = 94, BAP = 1.0), which is well separated to another one corresponding to *M. brandti* and *M. newtoni (*MP = 83, NJ = 98, ML = 93, BAP = 1.0*).*

- *Cricetulus griseus* and *C. barabensis* are strongly related (MP = 100, NJ = 100, ML = 94, BAP = 1.0) and associated to *C. longicaudatus* (MP = 100, NJ = 100, ML = 94, BAP = 1.0) whereas they are well separated from a group corresponding to *Tscherskia triton, Cricetulus migratorius, Cricetus cricetus* and *Allocricetulus eversmanni*.A summary of the Kimura 2 parameter (K2P) sequence divergence values is presented in table 2 for the three DNA regions, both within and between the studied genera. It indicates that the divergence between the genus *Phodopus* and the other Cricetinae genera is very high for the three DNA regions. An important level of genetic divergence is also observed between the genus *Mesocricetus* and *Cricetulus, Tscherskia,*

Cricetus and *Allocricetulus. P. roborovski* also appears highly divergent to the other studied *Phodopus* species suggesting an old separation between them. On the contrary, the genetic divergence is lower within *Mesocricetus* and *Cricetulus.*

Divergence time

The LRT rejected a clock for cyt*b* and 12S ($p<0.001$ and $p<0.05$ respectively) but not for vWF ($p=0.057$). However, the relative rate test indicated no significant rate heterogeneity between the three genes. Therefore, we consider the global clock strategy as potentially applicable in this case although its results should be interpreted with caution. To apply a global clock and to estimate times of divergence, we estimated the ML tree based on the combined data set with *Cricetomys gambianus* and *Nesomys rufus* as outgroup. The inferred maximum likelihood distances were used to estimate separation times. The ML distance between *Mus* and *Rattus* that diverged 12 MY ago is 0.233. The one between *Gerbillus* and *Tatera* that diverged 8 MY ago is 0.120. These values give a rate of 0.015-0.016 (*Mus*/*Rattus* and *Gerbillus*/*Tatera*) ML distance per million years. When this rate is applied to the different dichotomies within the Cricetinae, the following molecular datings are obtained: 8.5-9 MY for the separation between the genus *Phodopus* and the other Cricetinae genera and 7.6-8.1 MY for the separation between *Mesocricetus* and *Cricetulus, Tscherskia, Cricetus* and *Allocricetulus* (see table 3).The relaxed clock method produced time estimates overlapping with those based on a general clock assumption although demonstrating a tendency for deeper earlier splits (see table 3). *Phodopus* and *Mesocricetus* split at approximately 10.8-12.2 MY, while radiation within the *Cricetus*-related group started 6.5-7.5 MY ago.

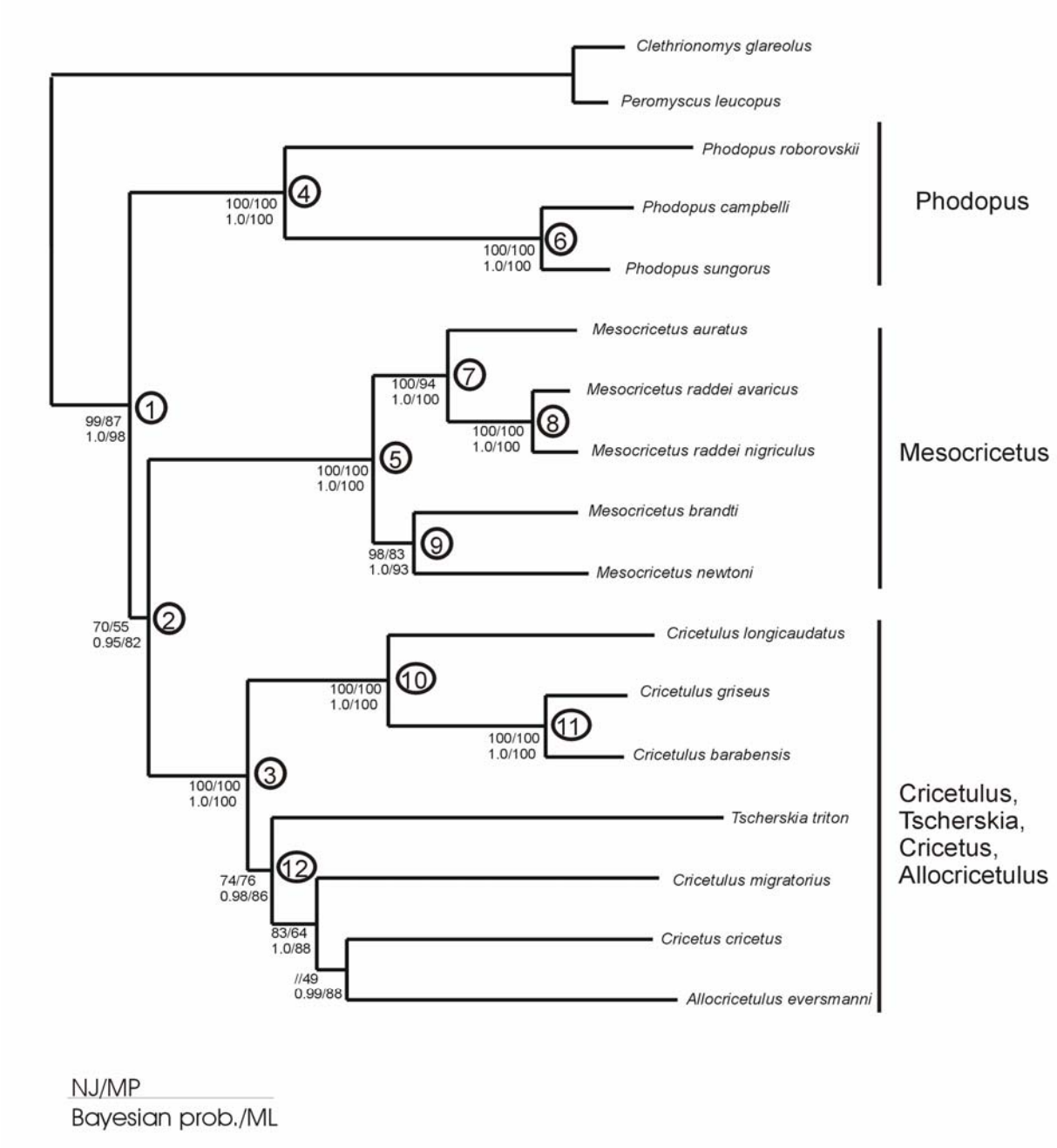

Fig. 2 Consensus tree obtained from the analysis of the concatenated genes for 15 taxa and 2559 positions, with the exclusion of transitions at third codon position for cyt*b*. Numbers inside opened circles correspond to the main nodes of the tree where divergence time estimates were calculated (see Table 3). *Clethrionomus glareolus* and *Peromyscus leucopus* are used to root the tree. For each node the different robustness indices are indicated as follows:

NJ Bootstrap support/MP Bootstrap support
Bayesian probabilities/ML Bootstrap support

Table 3 Time estimates for separation events within the subfamily Cricetinae (see Figure 2, for node numbers), calculated from molecular data. The numbers in bold correspond to calibration points: 12 MY for the separation between *Mus* and *Rattus* (Jaeger, Tong, and Buffetaut, 1986; Jacobs and Downs, 1994) and 8 MY for the separation between *Gerbillus* and *Tatera* (Tong, 1989). S.E. = Standard Error values provided by the maximum-likelihood analysis of Puzzle 4.0. "SC" refers to a standard clock and "RC" to a relaxed clock.

Separation events	Separation SC *Gerbillus/ Tatera*	S.E.	Separation SC *Mus/ Rattus*	S.E.	Separation RC	S.D.
Mus/ Rattus	15	0,4	**12**	/	12.9	0.6
Gerbillus/ Tatera	**8**	/	7,5	0.4	7.9	0.6
Phodopus/ other Cricetinae (node 1)	9.0	0.2	8.5	0.2	12.2	2.0
Mesocricetus/ Cricetulus, Tscherskia, Cricetus, Allocricetulus (node 2)	8.1	0.2	7.6	0.2	10.8	1.9
C. longicaudatus, C. griseus, C. barabensis/ T. triton, C. migratorius, Cr. cricetus, A. eversmanni (node 3)	6.2	0.2	5.8	0.2	7.4	1.5
P. roborovskii/ P. campbelli, P. sungorus (node 4)	5.2	0.3	4.9	0.3	6.9	1.5
M. brandti, M. Newtoni/ M. auratus, M. raddei (node 5)	2.7	0.2	2.5	0.2	2.7	0.8
P. campbelli/ P. sungorus (node 6)	0.9	0.1	0.8	0.1	1.0	0.5
M. auratus/ M. raddei (*avaricus, nigriculus*) (node 7)	1.5	0.1	1.3	0.1	1.2	0.5
*M. raddei avaricus/ M. raddei nigriculus (*node 8)	0.5	0.1	0.4	0.1	0.4	0.3
M. brandti/ M. newtoni (node 9)	1.8	0.1	1.7	0.1	1.7	0.6
C. longicaudatus/ C. griseus, C. barabensis (node 10)	3.1	0.1	2.9	0.1	3.1	0.9
C. griseus/ C. barabensis (node 11)	0.85	0.1	0.8	0.1	0.8	0.4
T. triton/ C. migratorius, Cr. cricetus, A. eversmanni (node12)	5.4	0.2	4.9	0.2	6.7	1.4

DISCUSSION

Phylogenetic structure and the evolution of higher taxa

A gene phylogeny derived from concatenated genomic sequences revealed a clear partition of Palaearctic hamsters into three main evolutionary entities: 1. *Phodopus* group, 2. *Mesocricetus* group, 3. *Cricetus*-related group (*Cricetus, Tscherskia, Allocricetulus, Cricetulus*). This grouping disagrees with previous systematic studies based on allozymes or morphological grounds (Flint, 1966; Fahlbusch, 1969; Corbet,

1978; Kartavtsev et al., 1984b; Carleton and Musser, 1984). However the same three clades are largely delimited on the basis of chromosomal data (Radjabli, 1975; Kartavtseva, 2001).

Molecular clock results derived from global and relaxed clock assumptions are rather similar except for more ancient splits such as node 1 and 2, where both estimates deviate by 2-3 MY from each other. Moderate rate heterogeneity among genes (e.g. mt versus nuclear) and lineages may account for that. However, both estimates exceed fossil datings which makes a judgment of the validity of the calculations impossible. We prefer to use the entire span of divergence times for further discussions.

Splits between major phylogenetic clusters (node 1, node 2) are rooted in the upper Miocene around 7.6 - 12.2 MY ago. Noteworthy, is the old age of the genus *Phodopus* (8.5 - 12.2 MY) because fossils were not reported before the Quarternary (Fahlbusch, 1969; Shaohua, 1984; Shaohua and Cai, 1991). Steppan et al. (2004) calculated a separation between *Phodopus* and *Mesocricetus* from four nuclear genes at 13.5 - 14.1 MY ago. In contrast with fossils, our datings indicate that by the end of Miocene the two *Phodopus* branches had been already distinct and, hence the ghost time for the genus covers the entire Pliocene. A lack of records and misidentification of fossils may account for the discrepancy between fossil and genetic data (Schaub, 1930; Shaohua and Cai, 1991).

Uncertainties concerning species identity as well as phylogenetic relationships between fossil and extant hamsters impede a clear correlation between the rise of major hamster taxa and specific geographical or climatic events in the past. However, our results show a good congruence between the appearance of major phylogenetic lineages during the Late Miocene and the fossil based description of a large number of Cricetine species in Europe and Asia (McKenna and Bell, 1997) from the middle and late Miocene epoch. A major reason explaining such Cricetinae diversification would be the spread of steppe and open woodlands due to increasingly drier climatic conditions 8 - 10 MY ago. In particular during the late Miocene many parts of Europe and Asia were characterized by a drastic faunal turn over in favour of open land species due to ongoing global changes in vegetation (Cerling et al., 1997). The same time period was also characterized by tectonic changes such as a further uplift of the Tibetian plateau (~10 MYA) providing physical barriers as well triggering climatic changes across Eurasia (Agustí et al., 1999; Fortelius et al., 2002). Most affected regions in Mongolia and China still harbour a significant number of hamster species

(see also Figures 1 – 3). It is reasonable to hypothesize that (Central?) Asian grasslands were the primary centre of diversification of major hamster taxa. However, some extinct genera which have been discussed as potential ancestors, in particular for the extant *Cricetus*-related clade, e.g. *Kowalskia* were also reported from the late Miocene in Europe (McKenna and Bell, 1997). Shaohua (1984) speculates a phylogenetic link between *Tscherskia triton* and the extinct genus *Kowalskia*, which is known from both Europe and Asia since the late Miocene (Rummel, 1998; Kowalski, 2001). It is well possible that divergence within the *Cricetus*- group took place within and outside the central Asian basin. Extant distributions give no clear signals as for instance *Cricetus cricetus* is mainly found in Europe and east of the Altai-Sajan whereas *Tscherskia* occurs in eastern China however, having recently become extinct in north-western Iran. The lack of *Cricetus* fossils in Mongolia and China as well as the fact that putative *C. migratorius* or *C. migratorius*-related fossils occured in Europe since the older Pleistocene (Jánossy, 1986; Storch, 1975) and perhaps the late Pliocene (De Bruijn et al., 1970; Niethammer and Krapp, 1982) could imply an evolutionary centre of these species in Europe.

Unfortunately, the lack of clearly specified fossils does not allow pinpointing an ecological or geographical event leading to the divergence of the *Phodopus* group from the remaining clades. Clearer is the situation for *Mesocricetus* hamsters although no putative ancestral hamster taxon could be identified so far. Oldest fossils related to the *Mesocricetus* lineage come from the Aegean region such as *M. primitivus* from Chalkidike (L-Miocene/E-Pliocene) (Vasileiadou et al., 2003) and Rhodos (upper Pliocene; De Bruijn et. al., 1970) and central Turkey (E-Pliocene; Sevket et al., 1998). The distribution of *Mesocricetus* fossils dating to the Miocene/Pliocene boundary as well as the fact that all fossils found so far roughly coincide with the genus' recent geographical range strongly favour an evolutionary origin in the eastern Mediterranean (Storch, 2004). There is a number of evidence supporting climatic change and the formation of steppe-like landscape following the raise of the Aegean and Anatolian land mass during the middle and late Miocene (Lüttig and Steffens, 1975).

The spread of deserts across Asia during the Pliocene has likely facilitated a further diversification within major hamster clades mainly triggering the radiation of *Cricetus*-related hamsters. This clade harbours most of the extant hamster species which are distributed from western Europe to eastern Asia indicating a relatively wide ecological

potential compared to the other main hamster lineages. Congruent with such a scenario is the appearence of hamsters of the *Cricetulus*-type in the Russian steppe since the middle Pliocene (Gromova, 1962).

Late Tertiary and Quaternary expansion led to longitudinal range splits and speciation

Some closely related hamster species have allopatric ranges in east-west orientation; *A. eversmanni/A. curtatus*, *Phodopus campbelli/P. sungorus*, *Cricetulus barabensis*-group (Figure 3). Such a longitudinal zonation is typical for the eastern part of Mongolia and China and adjacent Siberia and Kazakhstan where the Sajan-Altai mountains function as efficient gene flow barriers. This distribution of subdivided populations is comparable to the phylogeographic pattern of steppe plants (Franzke et al., 2004). A belt of steppe has repeatedly functioned as an expansion corridor from the central Asian plateau to the west. At times of favourable climatic conditions hamsters were able to pass (river valleys, e.g. Irtysch, Yennisej) or bypass the mountain area crossing Dsungaria (see Figure 3). Franzke et al. (2004) estimated a time of 0.5-1.1 MY for the divergence of population groups in the steppe plant *Clausia aprica* covering the discussed geographical area. A divergence time of 0.8 – 1.0 MY between *Phodopus campbelli* and *P. sungorus* falls exactly in this range implying a strong correlation between Quarternary expansion of steppe and migration of Asian hamster populations. Western range extensions during cooler periods of the Quarternary followed by a subsequent disintegration of populations due to deteriorating ecological conditions (cooling or warming maxima) were found to be a key mechanism for genetic subdivision in common hamster populations (Neumann et al., 2005) and most likely accounts for longitudinal speciation. Unfortunately, there are hardly genetic data for other steppe adapted mammals in the Sajan-Altai region to investigate the importance of such a speciation process. The spatial distributions of steppe lemmings *Eolagurus luteus* - *E. przewalskii* and feather-tailed jerboas *Stylodipus andrewsi* – *S. telum / S. sungorus* (see Pantelejev, 1999) are similar to that of the above mentioned central Asian hamsters and could be the result of a westward expansion.

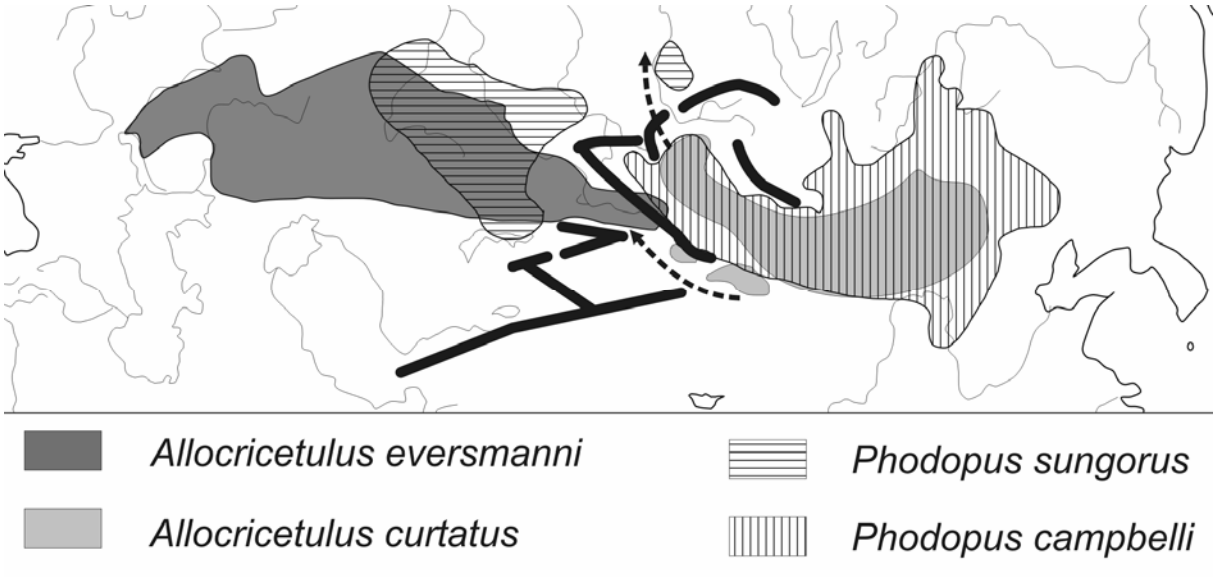

Fig. 3 Distribution areas of closely related sibling species: *Allocricetulus eversmanni*/ *A. curtatus* and *Phodopus sungorus*/ *P. campbelli*. Dark bars indicate mountain ranges of the Sajan-Altai. Arrows refer to potential migration routes during the Pleistocene.

Genetic evaluation of species systematic positions

Genus*: Phodopus*

The genus harbours small sized hamsters with peculiar adaptations to extreme temperatures such as hairy feet and special thermoregulation (Ross, 1994, 1995, 1998, Newkirk et al., 1998). Furthermore, *Phodopus* species are the only known hamsters showing high social tolerance and even biparental behaviour (Wynne-Edwards and Lisk, 1987). Controversial opinions exist about the validity of *Phodopus campbelli* which is sometimes considered to represent only a subspecies of *P. sungorus* (Flint, 1966; Honacki et al., 1982). Our phylogenetic analyses (see Table 2) indicate that the two dwarf hamsters are very closely related, e.g. for cyt*b*, they are only separated by 4.5% of genetic divergence. Both species have probably split 0.8 – 1.0 MY ago during the Pleistocene. Our dating correlates with the divergence time proposed by Lougheed et al., 2003; cited in Wynne-Edwards, 2003). The time estimate falls in a period of relative climatic instability with a short stadial (0.9 – 0.8 MY) interrupting two warming periods. Late Pleistocene remains of *Phodopus* hamster were reported from different places in Europe (Schaub, 1930; Rathgeber and Ziegler, 2003; but questioned in Gromov and Baranova, 1981) and Asia (Shaohua, 1984) implying a temporarily wider distribution. It is likely that *P. sungorus* populations are relicts of an old westward expansion during the Biharian stage of the Pleistocene presuming that *Phodopus* hamsters originated close to its recent main distribution area. Notable is the distinguished position of *P. roborovskii* which shows a higher degree of diver-

gence to *P. campbelli/sungorus* than found between some other acknowledged hamster genera. Cyt*b* distances measure 19.2±1.6%/18.0±1.6% what is above the average expected for intrageneric differentiation in small mammals (Bradley and Baker, 2001). Profound genetic differences between *P. roborovskii* and *P. campbelli/sungorus* go in line with a number of morphological characters dividing the species (Argyropulo, 1933; Vorontsov, 1960; Ross, 1994). *P. roborovskii* is not only the smallest of all hamsters but is also the only one which is adapted to extreme arid desert conditions living preferentially along sand dunes. Schmid et al. (1986) considered the karyotype of *P. roborovskii* to be more primitive than that of *P. campbelli* and *P. sungorus*. For those reasons we propose to place *P. campbelli* and *P. sungorus* in the genus *Cricetiscus* following Thomas (1917).

Genus: *Mesocricetus*

Four species have been accepted so far; *M. raddei*, *M. newtoni*, *M. brandti* and *M. auratus* (Wilson and Reeder, 1993). Sequence data confirm the validity of those species but revealed much greater genetic distances as expected from morphological data (Hamar and Shutowa, 1966; Yigit et al., 2000). *M. raddei nigriculus* appears genetically very closely related to *M. raddei avaricus*. Morphological differences between the two “subspecies” are therefore likely the result of ecological adaptations. The smaller *M. r. nigriculus* occurs in the ciscaucasian lowlands whereas *M. r. avaricus* is an element of the mountainous steppe regions in central Dagestan. *Mesocricetus* hamsters show mainly a vertical zonation of species ranges which are divided by mountain chains; the Caucasus and the Taurus. A separation of *Mesocricetus* hamsters into a cis- and a transcaucasian group as proposed by Hamar and Schutowa (1966) could no be confirmed. Reason therefore is the close association of the transcaucasian *M. auratus* with the ciscaucasian *M. raddei*. Current distribution areas of both species are highly isolated from each other (*M. raddei*-Caucasus, *M. auratus*-Aleppinian plateau) and account for range displacement during the Pleistocene (1.3 – 1.5 MY). M*esocricetus* hamsters of the *auratus*/*brandti*-type appeared in south-west Asia, the Aegaeis and the Caucasus region throughout the Pleistocene and since the lower Palaeolithicum in Transcaucasia (Vereshchagin, 1959; Storch, 1975; Tchernov, 1975; Güleç et al., 1999). Molecular clock estimates indicate a separation between the *M. brandti*/*M. newtoni* and *M. raddei*/*M. auratus* group during the upper Pliocene. It is well possible that the spread of dry steppe

habitats and tectonic changes supported an early split into a western (west/central Anatolia) and an eastern (Caucasian) *Mesocricetus* group. Vereshchagin (1959) suggested a penetration of the Caucasus by *Mesocricetus* hamsters as early as in the upper Pliocene. Disintegration of the eastern *Mesocricetus* group (*M. auratus/M. raddei*) may have occurred during the lower/mid Pleistocene a time with significant range extensions in *Mesocricetus* hamsters (Vereshchagin, 1959). Unfortunately, there are no reliable fossil data to time the arrival of *M. auratus* in its contemporary range but its most southerly boundary was reached about 40 KY ago (Tchernov, 1975). The separation of *M. brandti* and *M. newtoni* occurred probably during an early stage of the Pleistocene.

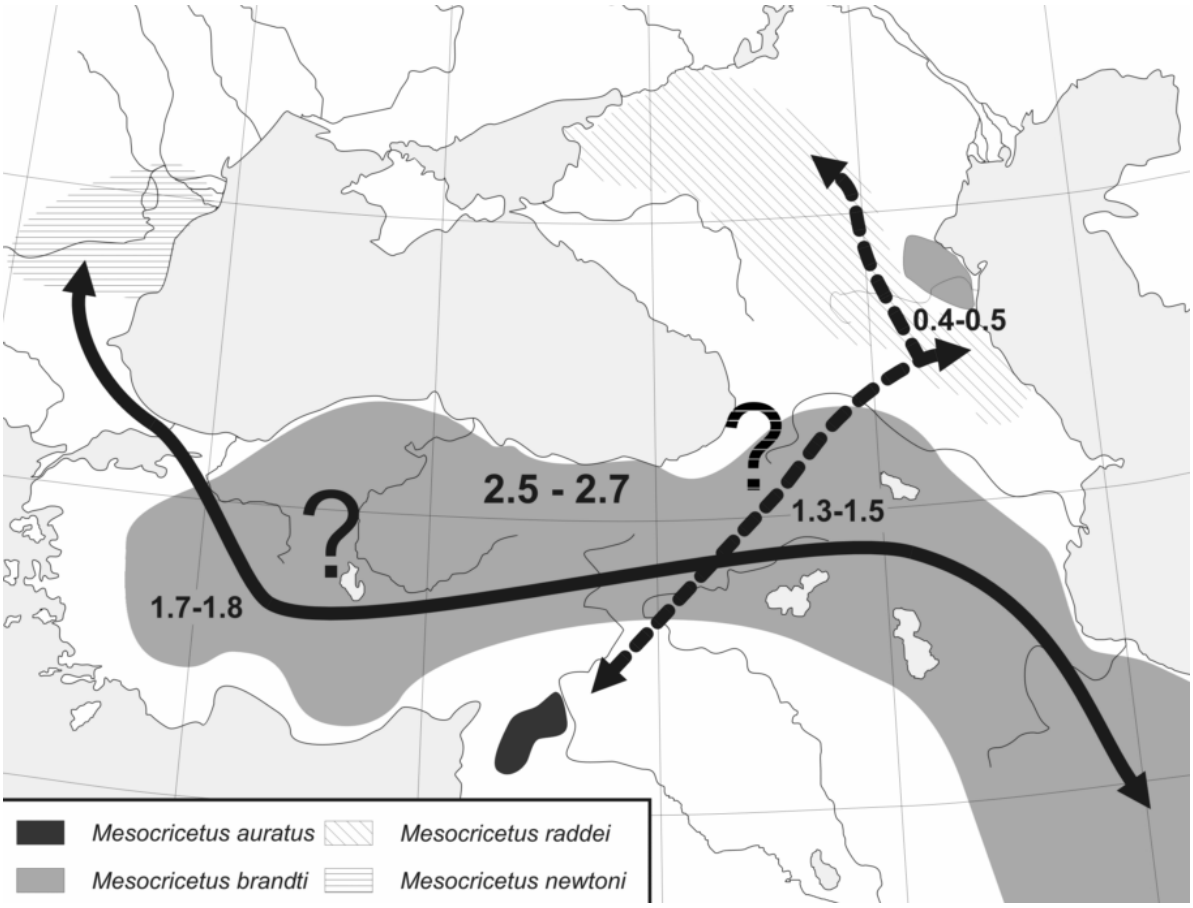

Figure 4 Distribution areas of *Mesocricetus* hamsters. Question marks indicate potential areas of evolution of the two main *Mesocricetus* grous. Arrows refer to range extensions. Numbers refer to calculated dates of divergence in million years.

Our time estimate (~1.7 – 1.8 MY) slightly predates middle Pleistocene fossil records (*M. cf. newtoni*) from Turkish Thrace (Santel and von Königswald, 1998). *M. newtoni* probably reached its contemporary range during the Holsteinium (Kowalski, 2001). Shifting altitudes and vegetation changes in the Caucasus, a further uplift of the Taurus as well as multiple transgressions of the Caspian, Black and Mediterranean sea

are very likely responsible for the relatively rapid radiation of *Mesocricetus* species during the Pleistocene. Noteworthy is that competition between *Cricetus* and *Mesocricetus* (Tchernov, 1975) could be an important factor which restricts the range of *Mesocricetus* in the north and west.

Genus: *Cricetulus*

Cricetulus is with eight (Panteleyev, 1998) or six species (Duff and Lawson, 2004) the largest hamster genus and provides most identification difficulties. Four species groups are easily recognized on morphological features including monotypic *migratorius* and *longicaudatus* groups, *barabensis*-group with four chromosomal forms (*barabensis, griseus, pseudogriseus, sokolovi*) the ranks of which are still disputed, and yet poorly known *kamensis* group treated as a subgenus *Urocricetus*. Our sample contained only four species *C. barabensis*, *C. griseus*, *C. longicaudatu*s and *C. migratorius*. The species identity of *C. griseus* is largely based on karyological grounds (Matthey, 1960) but its relevance is questioned (Král et al., 1984). Some authors consider *C. griseus* only as a subspecies of *C. barabensis* (Wilson and Reeder, 1993). The genetic distance obtained in the present study between *C. griseus* and *C. barabensis* falls in this last range (Table 2) and is similar to what is observed between *Phodopus campbelli* and *P*. *sungorus*. Differentiation of the *barabensis/griseus* group probably occurred in the late to middle Pleistocenic period due to the expansion/contraction of areas caused by changes in humidity levels. *C. longicaudatus* forms a stable, well-supported association with the two representatives of the *barabensis*-group. This outcome is not unexpected due to a high level of morphologic similarity between the two groups which are sometimes lumped into a single one (Pavlinov and Rossolimo, 1987). The most striking result is the lack of monophyly of the genus *Cricetulus*. The widespread *Cricetulus migratorius* stands outside the actual *Cricetulus* group and is more affiliated with *Cricetus* and *Allocricetulus*. As a consequence we propose to place the species in a new monotypic genus. At this point it should be mentioned that a preliminary study on the 12S of *C.* (*Urocricetus*) *lama* (Lebedev et al., 2003) identified the species as a potential sister genus of *Phodopus* or a representative of very basal hamster taxon. Summarizing all genetic evidence one has to conclude that the genus *Cricetulus* represents just a “dustbin” of small-sized hamsters with advanced dental morphology.

Genus: *Tscherskia*

Tscherskia triton does clearly not belong to the genus *Cricetulus*. It represents a separate lineage within the *Cricetus*/*Cricetulus* group which might be closer to *Cricetus* and *Allocricetulus*. The taxon evolved at the end of the Miocene/early Pliocene. Shaohua (1984) postulates *T. triton* as a descendant of *Cricetinus* (*Tscherskia*) *varians* which is according to him very likely related to *Kowalskia*. The relationship of *Tscherskia* with *Cansumys* remains to be established. The latter genus was for long treated as a synonym of *Tscherskia*. However, no genetic data on *Cansumys* is available thus far.

Genus: *Cricetus*

The common hamster *Cricetus cricetus* is the only hamster genus of the *Cricetus*-related clade, which lives exclusively west of the Sajan-Altai. Typical *Cricetus* hamsters were found from the beginning of the Pleistocene (Niethammer and Krapp, 1982; Hir, 1997) but only C*ricetus cricetus* survived areal shifts and local extinction during glacial oscillations. Mein and Freudenthal (1971) questioned the generic identity of Pliocenic and Quarternary *Cricetus* species. DNA data place the origin of the genus *Cricetus* in the upper Pliocene what recommends a re-evaluation of these records. In fact, Topachevski and Skorik (1992) place *Cricetus kormosi* and some other *Cricetus* forms from the late Miocene to a new genus *Pseudocricetus*. Freudenthal et al. (1998) describe European *C. kormosi* or *C. cf. kormosi* from the late Miocene and Pliocene as *Apocricetus alberti*. Both views are concordant with our molecular findings.

Genus*: Allocricetulus*

The genus harbours two closely related species *A. eversmanni* and *A. curtatus*. DNA sequence analysis of *A. eversmanni* confirms the validity of an own genus and places the species in close neighbourhood to *Cricetus*. Published fossil records date exclusively from the Pleistocene (Flint, 1966). Our clock estimates place the origin of the genus already in the Pliocene.

CONCLUSIONS

The presented DNA based phylogeny of the subfamily Cricetinae provides significant new insights in the evolutionary history and systematics of old world hamsters. Our study evidenced that the Cricetinae are separated in three main genetic lineages which diversified in two different periods, firstly during the middle/end of Miocene, and secondly during the latest Miocene/Pliocene. Within-genus phylogenies root mainly in the Pliocene/Pleistocene boundary. The diversification is probably the result of important global climate changes which appeared during these periods all over the Palearctic region. Such large scale climatic events include above all the formation of extensive grass lands during dry parts of the late Tertiary as well as the further uprise of Asian mountain chains. During the Pleistocene, longitudinal range expansions led to the evolution of closely related sibling species west of their native central Asian range. Problematic for the systematics point of view are the varying levels of sequence divergence within and between genera. They hardly justify the maintenance of a single genus *Phodop*us or vice versa the establishment of different genera within the *Cricetus*-related group.

However, our genetic data can only provide a framework for further investigations as not all currently acknowledged species could be included. In particular a comprehensive taxonomic revision of the *Cricetulus* hamsters is desirable. The relatively poor fit of genetic and fossil data requires an urgent reevaluation of fossil identifications to verify molecular clock estimates. However, the presented data will now allow examining the ecological and climatic circumstances which formed the observed phylogenetic pattern not only on a global but also on a regional scale, e.g. the importance of the Sajan-Altai barrier for the population exchange between the central Asian plateau and the adjacent western lowlands. Hamsters can thereby provide an important reference group for other Eurasian steppe taxa. A future study should include the missing species mentioned to confirm our different hypotheses and to provide a definitive idea about the phylogeny of this still underrepresented Muroid subfamily.

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3. Genetische Diversität, Populationsstruktur und Phylogeografie des Feldhamsters (*Cricetus cricetus*)

3.1. Zur Biologie des Feldhamsters

Der Feldhamster ist mit 200 – 600 g der größte Vertreter der Cricetinae (Abb. 2). Sein Verbreitungsgebiet reicht vom Jenissej in Westsibirien bis nach Belgien und den Niederlanden (Niethammer 1982). Feldhamster sind ein typisches Element der Offenlandschaften (Steppe, Waldsteppe) und kommen in Mitteleuropa vor allem auf Agrarflächen vor. Im östlichen Kernverbreitungsareal werden aber auch Auen und krautreiche Wälder besiedelt, wobei der Hamster im Alatau bis auf Höhen von mehr als 2000 m vordringen kann (Berdyugin und Bolshakov 1998). Die relativ komplexen Baue werden bevorzugt in Löss oder tiefen lehmreichen Böden angelegt.

Abb. 2 Feldhamster *Cricetus cricetus* (Foto: K. Neumann)

Feldhamster sind weitgehend solitär lebende Nager, doch ergaben neuere Untersuchungen in Sachsen-Anhalt (Kayser 2003), dass Interaktionen zwischen Individuen sehr häufig sind. Eine noch laufende Studie auf einer Versuchsfläche in Sachsen-

Anhalt zeigt, dass die Aktivität adulter Feldhamster vor allem in den Morgen- und Abendstunden liegt (Mundt, Dissertation in Vorbereitung). Es gibt keine ausgeprägte Nachtaktivität. Eine ähnliche bimodale Aktivitätsverteilung mit Maxima am Morgen und in der Abenddämmerung wurde auch bei anderen mitteleuropäischen Populationen belegt (Nechay et al. 1977; Wendt 1989a; Kayser 2003). Im Gegensatz dazu werden ausgedehnte Streifzüge bis 2,5 km während der Nacht und ganztägige Aktivität in der Fortpflanzungsperiode für russische Hamster angeben (Berdyugin und Bolshakov 1998). Relative einheitlich sind die Befunde zum Raumnutzungsverhalten. Männliche Feldhamster haben größere Streifgebiete als Weibchen und halten sich öfter außerhalb und in größerer Entfernung der Baue auf (Karaseva 1962; Weinhold 1998; Kayser 2003). Die Streifgebiete der Männchen überlappen sich häufig mit denen mehrerer Weibchen (Weinholdt 1998; Kayser 2003). Die Nahrung der Hamster besteht vor allem aus grünem Pflanzenmaterial und Samen. Der Anteil tierischer Kost schwankt entsprechend seiner Verfügbarkeit und kann zwischen 10 und 20 % betragen. Im Spätsommer werden Wintervorräte angelegt, die in Extremfällen weit mehr als 30 kg betragen (Wendt 1989b; Berdyugin und Bolshakov 1998). Die Tiere sind fakultative Winterschläfer, deren Schlafintervalle (ca. 7 - max. 15 Tage) von Aktivitätsphasen unterbrochen werden (Wendt 1984; Wollnik und Schmidt 1995; Wassmer 2004). Feldhamster erscheinen in Mitteleuropa im März-April mit dem Beginn der Fortpflanzungsperiode, wobei Männchen eher aktiv werden als Weibchen. Das Paarungssystem ist promiskuitiv. Genetische Analysen (Neumann unpubliziert) haben gezeigt, dass im Fall von zwei aufeinander folgenden Würfen eines Weibchens (Hakel/Sachsen-Anhalt, 2000), diese jeweils von einem anderen Männchen stammten. In einem Wurf (4 Junge) aus der Umgebung von Brno (Tschechische Republik, 1999) konnte eine multiple Vaterschaft nachgewiesen werden (2 Väter). Die Angaben zur Tragzeit sind uneinheitlich. Nach Mohr et al. (1973) beträgt sie 15,5 - 17 Tage, wogegen Petzsch (1950) von 19 - 20 Tagen ausgeht. In Mitteleuropa und Russland kommen in der Regel 1 - 2 Würfe vor, während für das östliche Verbreitungsareal und den pannonischen Raum bis zu 3 Würfe belegt sind (Niethammer 1982; Nechay et al. 1977; Nechay 2000). Hamster erreichen die sexuelle Reife meist erst nach dem ersten Winter, aber es gibt Hinweise, dass junge Weibchen bereits im Jahr der Geburt reproduzieren können (Vohralík 1974; Nechay et al. 1977). Die Wurfgrößen liegen zwischen 3 und 11 Tieren (Vohralík 1974), sind mitunter aber auch höher. Das enorme Reproduktionspotential führt zu sehr dynamischen Popula-

tionsentwicklungen (Grulich 1980) und in günstigen Jahren zu Massenvermehrungen (Grulich 1986; Nechay et al. 1977).

3.2. Bestandssituation und Schutzstatus in Europa

Als Steppenart trat der Feldhamster vor allem während der kühleren und gemäßigten Abschnitte des Pleistozäns auf (Storch 1974; Kowalski 2000). Dagegen verursachten die Kältemaxima der Glaziale aber auch sehr warme Abschnitte, z.B. im Atlantikum und Subatlantikum des Holozäns, deutliche Arealverluste (Markova et al. 1995; Spitzenberger und Bauer 2001) für die Art. Eine letzte Ausbreitungsphase in Richtung Westen fand im Zuge der neolithischen Rodungen in Europa statt (Niethammer 1982; Clason 1999). Die heutige Verbreitung wurde somit wesentlich durch die fortschreitende anthropogene Landwirtschaft bestimmt. Seit Mitte der 80er Jahre des 20. Jahrhunderts kam es zu bedeutenden Bestandsrückgängen insbesondere im westlichen Mitteleuropa (Libois und Rosoux 1982; Baumgart 1996; Backbier und Gubbels 1998), aber auch im osteuropäischen Raum sind Rückgänge zu verzeichnen (Markov 1998; Murariu 1998; Nechay 2000). Als entscheidender Auslöser für den teilweise dramatischen Zusammenbruch der Feldhamsterbestände gilt die umfassende Intensivierung der Landwirtschaft mit veränderten Bearbeitungsmethoden, Anbaukulturen und Fruchtfolgen (Pelzers et al. 1984; Backbier und Gubbels 1998; Kayser 2003). Inzwischen wurden nationale Schutzmaßnahmen für den Feldhamster in verschiedenen europäischen Ländern getroffen. In Deutschland wurde die Art in die Roten Listen der einzelnen Bundesländer aufgenommen. Die Rote Liste der Bundesrepublik Deutschland führt den Feldhamster als stark gefährdet (Binot et al. 1998). Europaweit ist der Hamster durch die Berner Konvention und die FFH-Richtlinie geschützt (Mitchell-Jones et al. 1999). Trotz intensiver Bemühungen sind einige westliche Populationen vom Aussterben bedroht oder schon weitestgehend verschwunden, wie in den Niederlanden. Hier läuft seit 1999 ein Gefangenschafts-Zuchtprogramm für den Feldhamster und mit den ersten Wiederansiedelungen wurde 2002 begonnen (Krekels 1999). Sämtliche genetischen Begleituntersuchungen für das niederländische Zuchtprojekt werden in Halle (unter meiner Leitung) durchgeführt. Im Jahre 2005 wurde ein Zuchtprogramm für den Feldhamster in Deutschland beschlossen.

3.3. Zur genetischen Diversität und Differenzierung europäischer Feldhamsterpopulationen

Der Verlust genetischer Variabilität durch stark verringerte Individuenzahlen (Flaschenhals, Gründereffekt) und eine geschlossene Populationsstruktur stellt ein wesentliches Aussterberisiko für Wildpopulationen und Gefangenschaftszuchten dar (Frankel 1983; Frankel und Soulé 1991; Crnokrak und Roff 1999; Hildner 2003). Eine Erhöhung des Homozygotiegrades kann zu Inzuchtdepressionen führen. Gründe dafür sind die Expression rezessiver nachteiliger Allele oder die nicht mehr ausbalancierten Effekte überdominanter Loci (Frankel 1983; Reed et al. 2003). Gleichzeitig beeinträchtigt eine reduzierte Anzahl adaptiver Allele das Vermögen eines Organismus, auf Änderungen seiner Umwelt zu reagieren. Studien belegen, dass eine Reduktion genetischer Variabilität mit verminderter Überlebenswahrscheinlichkeit, kleineren Wurfgrößen und verzögerter Jungenentwicklung assoziiert sein kann (Madsen et al 1996; Dietz et al. 2000; Meagher et al. 2000). Es konnte ein deutlicher negativer Zusammenhang zwischen genetischer Variabilität und Populationswachstum hergestellt werden (O'Brien 1985; Saccheri et al. 1996; Hildner 2003). Allerdings gibt es auch Beispiele, wie Mauritiusfalke *Falco punctatus* (Groombridge et al. 2000) und skandinavische Biber *Castor fiber* (Ellegren et al. 1993), bei denen Populationen trotz hoher genetischer Homogenität eine gute Bestandsentwicklung genommen haben. Inzuchteffekte sind häufig maskiert und werden erst in einer entsprechenden Umweltsituation wirksam, wie z.B. intra-spezifische Konkurrenz (Meagher et al. 2000; Joron und Brakefield 2003) oder Erregerresistenz (Kappe et al. 1997).

Eine Reihe von Arbeiten beschäftigt sich mit der phänotypischen Variabilität und Populationsstruktur (Grulich 1987a, 1987b; Petzsch 1961; Pradel 1985; Kayser und Stubbe 2000) bzw. mit taxonomischen Aspekten des Feldhamsters (Niethammer 1982; Nechay et al. 1977; Mitchell-Jones et al. 1999; Nechay 2000). Die Studien ergaben eine relativ hohe Variabilität morphologischer Merkmale, wie z.B. Fellfarbe oder Körpermaße, innerhalb und zwischen Populationen aufgrund ausgeprägter Geschlechtsdimorphismen als auch in Abhängigkeit von Populationsdichten. Spitzenberger (2001) schlussfolgerte, dass eine Gliederung europäischer Feldhamster aus diesen Gründen nicht möglich ist. Trotz der einschränkenden Bewertung morphologischer Charakteristika wird die Existenz zweier Unterarten *C. c. cricetus* (Osthamster) und *C. c. canescens* (Westhamster) für Mitteleuropa angegeben (Mitchell-Jones et al. 1999). Berdyugin und Bolshakov (1998) listen weitere Unterarten für

Osteuropa und Asien, die im Allgemeinen nicht anerkannt werden (Niethammer 1982, Nechay 2000). Die im Zusammenhang mit den dramatischen Bestandseinbrüchen in West- und Mitteleuropa eingeleiteten Schutzmaßnahmen der letzten Jahre erforderten eine umfangreiche Kenntnis der genetischen Variabilität und Populationsdifferenzierung. Im Extremfall sank die Feldhamsterpopulation in Limburg/Niederlande auf wenige Individuen. Fünfzehn Tiere wurden 1999 bei Heer eingefangen und bilden die Basis eines umfangreichen Feldhamster-Zucht und Wiederansiedelungsprogramms in den Niederlanden (Krekels 1999, Jansman et al. 2003; Abb. 3). Aus diesen Gründen entstanden eine Reihe von Projekten im Auftrag des Kultusministerium Sachsen-Anhalts und der niederländischen Umweltorganisation *ALTERRA Green World Research*. In den **Publikationen II: „Polymorphic microsatellites for the analysis of endangered common hamster populations (*Cricetus cricetus* L.)“ und III: „Multiple bottlenecks in threathened western European populations of the common hamster *Cricetus cricetus* (L.)**“ werden die Entwicklung eines genetischen Markersystems und erste Erkenntnisse zur genetischen Diversität von Feldhamsterpopulationen dargelegt. Es wurden zwei unterschiedliche Markersysteme ausgewählt: die erste hypervariable Domäne der nichtkodierenden Kontrollregion als mitochondrialer DNA-Abschnitt und nukleäre Mikrosatelliten. Mitochondriale DNA wird fast ausschließlich (Kondo et al. 1990; Gyllensten et al. 1991) mütterlich vererbt. Die relative hohe Sequenzvariabilität (insbesondere der Kontrollregion) erlaubt ein hohes Maß an Populationsdifferenzierung. Da mitochondriale DNA, mit wenigen Ausnahmen (Maynard Smith und Smith 2002), keiner Rekombination unterliegt, ist es möglich, historische Beziehungen (Stammbäume oder Netzwerke) zwischen den mütterlichen Linien verschiedener Populationen herzustellen (Avise 1994). Nukleäre Mikrosatelliten sind das dominierende Markersystem für populationsgenetische Fragestellungen (Bruford und Wayne 1993; Jarne und Lagoda 1996; Zhang und Hewitt 2003). Sie sind häufig das Mittel der Wahl bei der Untersuchung von Populationsstrukturen, genetischer Variabilität und für Verwandtschaftsanalysen. Es sind DNA-Segmente, die in der Regel aus 2 - 6 Basen langen Motivwiederholungen bestehen und co-dominant vererbt werden. Die Länge von Mikrosatellitenloci ist hochvariabel mit Mutationsraten von 10^{-2} – 10^{-6}, wobei die meisten Mutationen einem “step-wise mutation“ – Prozess folgen. Mikrosatelliten gelten als weitestgehend neutrales System. Dies kann aber nur mit Einschränkungen gesehen werden.

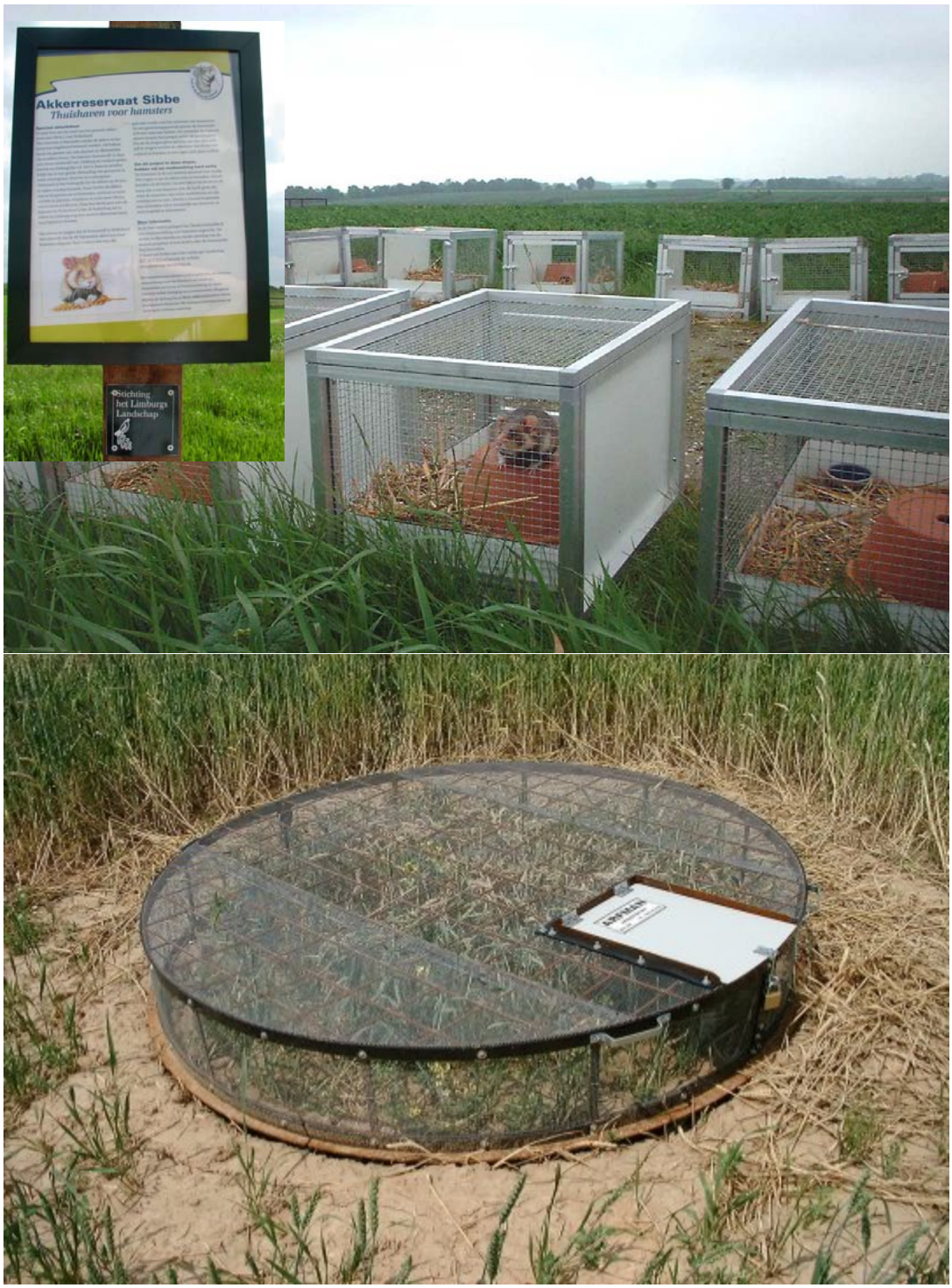

Abb. 3 Wiederansiedelung des Feldhamsters *Cricetus cricetus* in Sibbe/Niederlande (oben). Weiblicher Feldhamsterbau mit provisorischer Sicherung gegen Prädatoren (unten). Fotos: H. Jansman

Es gibt Hinweise für Mechanismen, die das überproportionale Längenwachstum von Mikrosatellitenloci beschränken (“allele size constraint“, Garza et al. 1995).

Weiterhin wird das hohe Maß an Sequenzkonservierung bei einigen (wenigen) Loci (FitzSimmons et al. 1995) mit positiver Selektion in Verbindung gebracht. Mikrosatelliten können auch funktionelle Aufgaben erfüllen. Extreme Beispiele sind Erkrankun-

gen, die auf Triplet-Expansionen zurückgehen. Häufig angewendete Dinukleotid-Marker, wie CA/TG können Z-DNA-Strukturen bilden, die Promotor-Aktivität verhindern können z.B. Nucleolin-Gen (*Ncl*) der Ratte (Rothenburg et al. 2001). Bestimmte Längenvarianten von Dinukleotidrepeats in der 5'-Region des Vasopressin 1α – Rezeptors modifizieren die Expression des Gens in *Microtus* (Hammock and Young 2005). Die hohe Mutabilität von Mikrosatelliten führt zu einem gewissen Grad an Längen-Homoplasie, d. h. Allele mit gleicher Größe sind nicht identisch hinsichtlich ihrer Abstammung (Estoup et al. 2002). Das erschwert die Erstellung von Genealogien, insbesondere wenn größere Zeitabschnitte betrachtet werden. Ein wesentlicher Nachteil von Mikrosatelliten ist ihre relativ hohe Artspezifität, d.h. für die überwiegende Anzahl von Nichtmodelltierarten müssen geeignete Loci erst isoliert und getestet werden. Im Rahmen der Habilitation wurden zwei Methoden zur Identifizierung von Mikrosatelliten angewendet. Methode 1 basiert auf dem herkömmlichen Screening einer partiellen genomischen Bank von größenselektierten DNA-Fragmenten mit entsprechenden Gensonden (Neumann und Wetton 1995). Methode 2 nutzt eine modifizierte "enrichment"-Technik, bei der DNA-Segmente mit bestimmten Mikrosatelliten-Motiven selektiv über Hybridisierungs- und PCR-Schritte angereichert werden (Ostrander et al. 1992, siehe auch Publikationen 1 - 4).

Publikation II „Polymorphic microsatellites for the analysis of endangered common hamster populations *Cricetus cricetus* (L.)" präsentiert ein polymorphes Mikrosatellitensystem, welches sich ausgezeichnet für die Quantifizierung der genetischen Variabilität von Feldhamsterpopulationen eignet und eine Differenzierung von Populationen zulässt. Der Populationsvergleich zeigt, dass westliche Feldhamster aus Frankreich, Belgien und den Niederlanden eine deutlich niedrigere genetische Variabiliät aufweisen als Hamster aus Sachsen-Anhalt/Deutschland. Große genetische Distanzen (*Fst*-Werte) zwischen geografisch nahe gelegenen Populationen (u.a. Limburg/Niederlande-Flandern/Belgien) zeigen den hohen Isolationsgrad westlicher Populationen. Ein wesentlicher Aspekt der Studie ist der Vergleich von niederländischem Museumsmaterial (1926 - 1980) mit der letzten rezenten Population in Heer. Er beweist, dass mindestens die Hälfe an Mikrosatellitendiversität in den letzten Jahrzehnten verloren ging. Eine Parallelstudie zur MHC-Variabilität (Smulders et al. 2003) der niederländischen Hamster zeigt einen noch dramatischeren Verlust von DRB-Exon 2-Polymorphismen. Die genetischen Daten belegen auch, dass der Polymorphiegrad der niederländischen Feldhamster-Zuchtpopulation im Bereich der La-

borpopulationen von Nagetieren mit relativ hohem Inzuchtcharakter liegt (z.B. Mongolische Wüstenrennmaus, Neumann et al. 2001).

Die Publikation III „Multiple bottlenecks in threatened western European populations of the common hamster *Cricetus cricetus* (L.)“ liefert nicht nur eine erweiterte Diversitätsstudie, die eine größere Anzahl von europäischen Feldhamsterbeständen beinhaltet, sondern beschäftigt sich auch mit möglichen Ursachen für die beobachtete Populationsstruktur in West- und Mitteleuropa. Rezente Feldhamster der westlichen Population aus Deutschland, Frankreich, Belgien und den Niederlanden zeigen ähnliche Allelfrequenzen an Mikrosatellitenloci und die gleichen dominierenden mitochondrialen Haplotypen (Kontrollregion). Gleiches gilt für museales Balgmaterial der letzten 70 Jahre aus Belgien und den Niederlanden. Diese genetische Ähnlichkeit beweist, dass die reduzierte Variabilität westlicher Hamster bereits auf einen gemeinsamen historischen Flaschenhals zurückgeht und nicht allein mit derzeitigen Bestandseinbrüchen erklärt werden kann. In diesem Zusammenhang entspricht die westliche Hamsterpopulation dem klassischen “leptocurtic dispersal“-Modell (Ibrahim et al. 1996; Hewitt 1999). Die Wiederbesiedlung von Arealen nach einer klimatisch ungünstigen Periode erfolgt dabei durch vorauseilende kleinere Populationen. Starke Driftwirkung innerhalb der Gründerpopulationen und Isolation von der Hauptpopulation führen dann zur Etablierungen einer neuen, genetisch ärmeren Randpopulation. Alternativ dazu könnte eine historische Klimaverschlechterung eine Verkleinerung des Areals zur Folge gehabt haben, in deren Zuge nur eine Reliktpopulation im Westen überlebte und später wieder expandierte. Beide Szenarien, die des Gründereffektes und des Flaschenhalses, lassen sich derzeitig nur schwer überprüfen. Fossiliendaten sprechen aber eher gegen ein Überleben der westlichen Population während des glazialen Maximums (Storch 1974, 1987).

Die Studie zeigt anschaulich das Aussagepotenzial beider angewendeter Markersysteme. Die Verteilung von Mikrosatellitenallelen bestätigt die starke Isolation westlicher Populationen und gibt Hinweise für negative Populationsschwankungen in den ostdeutschen Beständen. Prozesse, die wahrscheinlich innerhalb sehr kurzer Zeit (wenige Jahrzehnte bis Jahrhunderte) stattfanden. Mitochondriale Daten dagegen beweisen einen hohen Grad an Homologie zwischen westlichen und ostdeutschen Hamstern, die wahrscheinlich auf einer vor ca. 10.000 - 15.000 Jahren stattfindende Ausbreitung des Feldhamsters in Richtung Westen beruht.

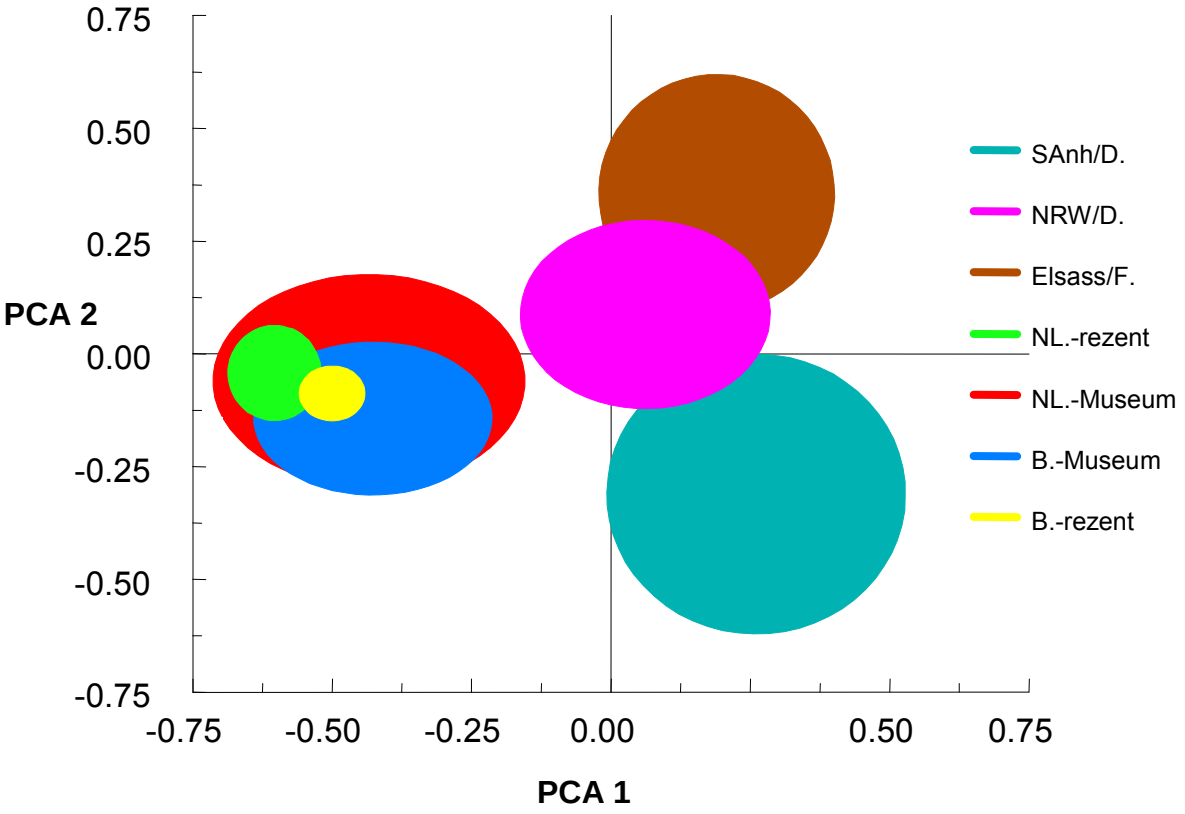

Abb. 4 Klusteranalyse basierend auf Nei's genetischer Standarddistanz kalkuliert von individuellen Mikrosatellitengenotypen (10 Loci) europäischer Feldhamster. Abkürzungen: SAnh/D,Sachsen-Anhalt/Deutschland; NRW/D, Nordrhein-Westfalen/Deutschland; F, Frankreich; NL, Niederlande; B, Belgien (nach Jansman und Neumann unpubliziert.)

Die beiden angeführten Studien beschreiben nicht nur die genetischen Konsequenzen der räumlichen Ausbreitung einer Kleinsäugerpopulation, sondern illustrieren anschaulich die zunehmende Fragmentierung westlicher Feldhamster (Abb. 4). Die Verwendung von Markersystemen mit unterschiedlichen Mutationsraten und effektiven Populationsgrößen, sowie der Vergleich mit musealem Material erlaubt eine bessere zeitliche Einordnung der Prozesse, die zur rezenten genetischen Diversität der untersuchten Populationen beitrugen. Daneben haben die gewonnenen Erkenntnisse einen direkten Einfluss auf laufende Schutzprogramme. Die geringe genetische Variabilität der niederländischen Reliktpopulation führte bereits zu einer Erweiterungen des Zuchtprogrammes in den Niederlanden, um mögliche Inzuchtprobleme im Laufe der Zeit zu vermeiden. Inzwischen wurden weitere Zuchtlinien unter Einbeziehung von belgischen Hamstern und Tieren aus Nordrhein-Westfalen (Deutschland) etabliert. Tatsächlich besitzen die neuen (genetisch etwas variableren) Zuchtpopulationen eine mit 6,5 – 7 Jungen höhere durchschnittliche Wurfgröße als die die rein niederländische Zuchtlinie mit 5 Jungen (La Haye persönliche Mitteilung). Das gesamte

niederländische Zucht- und Wiederansiedelungsprogramm unterliegt einem kontinuierlichen genetischen Monitoring auf der Basis des hier dargelegten Mikrosatellitensystems.
Seit 2005 werden sämtliche Jungtiere der niederländischen Gefangenschaftszuchten mit Hilfe von 11 Mikrosatelliten genetisch charakterisiert. Sowohl belgische als auch deutsche Feldhamster zeigen einige wenige charakteristische Allele. Diese werden als diagnostische Marker eingesetzt, um den Reproduktionserfolg der Zuchtlinien nach deren Freisetzung 2006 zu erfassen. Damit soll geklärt werden, ob sich die Zuchtlinien auch im Freiland hinsichtlich ihrer reproduktiven Fitness unterscheiden. Dieses Forschungsprojekt läuft im Auftrag von *ALTERRA* Green World Research Wageningen/Niederlande.

3.3.1. Polymorphic microsatellites for the analysis of endangered common hamster populations (*Cricetus cricetus* L.)

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Common hamsters (*Cricetus cricetus*) are characteristic mammals of open landscape in Europe. This semi-fossorial rodent inhabits steppe and cultivated fields with deep loess and clay-rich soils. Apart from a main distribution area in central Europe there are only a few isolated populations in western Germany, France, Belgium and the Netherlands (Niethammer 1982). These remnant areas represent the western distribution edge of the species. Formerly abundant, the common hamster experienced a rapid decline in its western range during the last thirty years. Growing infrastructure and changes in the agricultural cultivation scheme are the presumed reasons. In some countries, such as the Netherlands, the entire population was reduced to only a few individuals (Backbier and Gubbels 1998). As a consequence the common hamster is now protected under the EU habitat directive.

The dramatic situation of the common hamster in western Europe urged some countries to prompt captive breeding and reintroduction programs to prevent their local hamster populations from extinction (Kreekels 1999). Such an initiative was started in 2000 in the Netherlands.

As part of extensive conservation efforts we conducted a study to examine the genetic diversity of threatened common hamster populations. Information on the genetic resources of highly vulnerable populations and their potential effects is essential to develop successful species management plans, including captive breeding. Here we report the development of a microsatellite marker set for the common hamster and present first results concerning the genetic variability of these endangered populations. The isolation of microsatellites followed a protocol described in Neumann et al. (2001).

Die Markerentwicklung wurde durch mich durchgeführt. H. Jansman stellte DNA-Isolate der niederländischen und belgischen Hamster zur Verfügung. Die Fragmentanalysen wurden im eigenen Labor und in Wageningen durchgeführt. Die Auswertung der Populationsdaten und die Erstellung des Manuskripts erfolgte durch mich.

A genomic DNA library was established from six unrelated common hamsters by cloning doubly digested DNA-fragments (*Alu* I / *Hae* III, 0.3 – 0.8 kb) into XL - 1 Blue MRF´ cells (Stratagene). Approximately 1,200 recombinants were screened with a cocktail of four oligonucleotides $(CA)_{15}$, $(TC)_{15}$, $(GATA)_5$ and $(GAAA)_4$. Sixty positive clones were sequenced of which 38 contained uninterrupted arrays of more than 10 repeats. Primers were designed for 20 loci using OLIGO 5.0 (MedProbe, Norway). After testing, 11 microsatellites were selected for further analyses (Tab. 1).

Hamster genomic DNA was isolated from frozen and ethanol-fixed tissues such as ear (live trapped hamsters) and liver (mainly road victims) using an E.Z.N.A. Tissue DNA Kit II (Peqlab Biotechnology GmbH). Hair and skin samples from museum specimens were extracted with a DNAeasy Tissue Kit, (Qiagen Inc.) in a separate lab facility under a flow box fitted with an UV-light decontamination system (KR-130s BIOWIZARD, Kojair). Precautions against contamination included the use of separate autoclavable pipettes and filter tips. Negative controls were processed during the entire isolation procedures and subsequent PCR amplifications.

PCR was performed using the Ready-To-Go-system (Pharmacia). Twenty pmol of each primer and 0.01 - 0.1 µg of genomic DNA were added to a total volume of 25 µl. After an initial denaturation step of 180 sec. at 94°C, the amplification proceeded for 30 - 35 cycles as follows; 60 sec. at 94°C, 60 sec. annealing at 54°C for all primer combinations and 120 sec. at 72°C (Thermocycler UNO II, Biometra). PCR products were electrophoresed through 6% denaturing polyacrylamide gels on an A.L.F. express II (Amersham Pharmacia Biotech) automated sequencing system using commercial length standards. For population comparisons we analysed sixteen individuals (8 males and 8 females) from the last active Dutch hamster population at Heer (Limb/NL). This small sample represents almost the entire remaining population in Limburg. One male was found dead in 1997. All other animals were trapped alive in 2000. Six animals were juveniles and probably the offspring of one of the captured females. Fourteen of these hamsters went into a breeding program. Furthermore, we analysed 34 museum skins (LMus/NL, collected between 1926 and 1980 at 12 different sites in Limburg/the Netherlands). The maximum distance between sites was 40 km. Twenty-three museum samples provided sufficient DNA quality for fragment analyses. As reference populations we chose two other isolated western European populations from Belgium (FlVa/B, n=14) and France (Alsa/F, n=50) which are geographically close to the Netherlands sites.

Table 1 Details of 11 microsatellite loci developed for the common hamster *Cricetus cricetus.* Repeat motifs refer to the dominant satellite sequence. Loci Ccrμ3 and Ccrμ6 harbour allele size variants which are not caused by simple changes of the presented repeat units. Allele size ranges, allele numbers and heterozygosity values derived from the population SAnh/G.

Locus	Repeat motif	Primers (5'- 3')	Annealing temp. (°C)	Allele size range	No of. alleles	H_E	H_O	EMBL Accession No.
Ccrμ3	$(GT)_{23}$	F: ATGGAATTCATGGATTTCTTT R: CCCTACACACCCAACCTTA	50-52	192-202 bp	6	0.411	0.440	AJ532553
Ccrμ4	$(GT)_{18}$	F: ATCCCAACCTAGCATTGTATT R: CTTTCATATGTGTGCCAAGAC	54	189-209 bp	9	0.791	0.840	AJ532554
Ccrμ6	$(CTTT)_{13}$	F: TAAGTAGCAATGGTTCCTAGTA R: TAGTTCTAGGACAGCCTCCAAT	54	150-274 bp	13	0.842	0.860	AJ532555
Ccrμ10	$(CA)_{23}$	F: CTGTGCATCTGTTTGTCTGT R: GGTCTTAAGAATCAGGTGTGTT	54	190-204 bp	8	0.775	0.720	AJ532556
Ccrμ11	$(GT)_{22}$	F: AGGCATTTGCCACTATCGT R: TGAGAGATCCAACTCCTTCAAG	54	97-121 bp	13	0.628	0.660	AJ532557
Ccrμ12	$(GT)_{24}$	F: TGTTGTAATGGGCAGAT R: TTGCTGTTAGTGTCTTTGA	54	104-114 bp	6	0.535	0.400	AJ532558
Ccrμ13	$(GT)_{17}$	F: CTCAGAAACACAGCACAC R: GAGAATCATTTAGGCACAC	52	106-128 bp	4	0.650	0.680	AJ532559
Ccrμ15	$(CA)_{14}GA(CA)_{18}$	F: GCCCACTGCTACAAAAACTC R: GTTCATGAATGTTGTTAAATCTCT	54	224-240 bp	7	0.719	0.740	AJ532560
Ccrμ17	$(GT)_{20}(TG)_4(TG)_6$	F: GGTTATAAAGAGAAAAGACAAGAA R: GACTCCTGACATCCACCTC	52-54	237-249 bp	7	0.803	0.920	AJ532561
Ccrμ19	$(GT)_{23}$	F: AGTCATGTAAAGCCACTAAG R: ATTCAATTCAGCCACCAAAG	53	197-217 bp	10	0.813	0.740	AJ532562
Ccrμ20	$(TC)_{23}(CT)_9(CT)_6$	F: AAAAGCTGTTGATGACCACTT R: CATGGGGTATTTGGATGATTA	54	184-204 bp	9	0.808	0.880	AJ532563

Another reference population came from the eastern German province of Saxony-Anhalt (SAnh/G, n=50). This population lies within the main distribution area in central Europe. All samples were collected within the last ten years.

Genetic diversity measures such as allele number (A), observed (H_O) and expected heterozygosity (H_E) (Nei 1973) were computed in GENEPOP version 3.1 b (Raymond and Rousset 1995). The same program was used for calculating *Fst*-values, linkage disequilibrium and Hardy-Weinberg equilibrium (HWE). HWE tests were performed per locus for the population from Germany (SAnh) using a Markov chain method (Guo and Thompson 1992). The same probability test and a test of heterozygote deficiency were carried out for all populations over all loci. Allelic richness (RS) (El Mousadik and Petit 1996) was quantified in FSTAT (Goudet 1995).

All microsatellites (Tab. 1) worked well with fresh or ethanol fixed tissue material but some museum specimen provided amplification difficulties. In particular Ccrμ3 and Ccrμ6 were affected by poor DNA quality leading occasionally to PCR failure or spu-

rious bands. Such samples were excluded from analysis. Evidence for linkage was found for the locus combinations 10/12, 12/17, 11/20, 4/6 and 6/17 testing all pairs of loci across all populations. All significant values go back to non-random segregation of alleles in the population of Saxony–Anhalt. Significant deviations from HWE assumption were found for the Dutch museum population which can be explained by the heterogeneity of sample quality.

Table 2 Calculated means of allele number (A), allele richness (RS), expected (H_E) and observed heterozygosity (H_O) of six common hamster populations. Standard error numbers are given in brackets. *P* values refer to HWE probability tests.

Population	n	mean A	RS	H_E	H_O	p-value
SAnh (G)	50	7.273 (0.787)	4.170 (0.303)	0.707 (0.041)	0.716 (0.051)	ns
Alsa (F)	50	5.273 (0.832)	3.059 (0.404)	0.520 (0.077)	0.520 (0.077)	ns
Limb (NL)	16	1.636 (0.152)	1.506 (0.130)	0.180 (0.056)	0.159 (0.059)	ns
LMus (NL)	23	3.545 (0.368)	2.558 (0.408)	0.380 (0.077)	0.274 (0.054)	0.0002
FlVa (B)	14	2.273 (0.741)	1.937 (0.293)	0.228 (0.069)	0.157 (0.068)	ns

Allelic diversity varied greatly between populations (Tab. 2). As expected, hamsters from the largest population in Saxony-Anhalt proved most diverse (A=7.27, RS=4.17, H_E=0.71). Low diversity values were found in Dutch and Belgian hamsters (A=1.64 - 3.55, RS=1.51-2.56, H_E=0.18-0.38). Recent Dutch animals are fixed at 4 loci (Ccrμ3, Ccrμ4, Ccrμ13 and Ccrμ20). Genetic variability of historic samples from the Netherlands measured twice as high compared to the recent population. This is still significantly lower than values from Saxony-Anhalt ($p<0.001$, Mann-Whitney).

Genotyping of museum and hair samples is sometimes obscured by null alleles (Gagneux et al. 1997). To explore this problem we tested each locus for deviations from Hardy-Weinberg equilibrium due to heterozygote deficiencies.

Only the Dutch museum samples show significant deviations from expected values after corrections for multiple tests. Therefore, lower variability in recent French, Belgian and Dutch hamsters may not be caused by high proportions of null alleles. Some null alleles could be due to large allele size difference in heterozygotes (Wattier et al. 1998). Substantial allele size difference *Sd* was found at locus Ccrμ6 in the German population (Sd_{max}= 124 bp), while length variation in western hamsters was less dramatic (Alsa, Sd_{max}= 36 bp).

Large *Fst* values (SAnh/Alsa= 0.25 – Lim/FlVa=0.57) are consistent with the ex-

pected isolation among all studied common hamster populations. Our data revealed an alarmingly low degree of genetic polymorphism in the Dutch breeding stock. Whether this will have negative effects on the fitness of the newly established population should be studied in the future.

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3.3.2. Multiple bottlenecks in threatened western European populations of the common hamster *Cricetus cricetus* (L.)

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ABSTRACT

Common hamsters *Cricetus cricetus* (L.) show a highly fragmented distribution pattern across Europe. Over the last decades, human influence caused significant population declines in particular at the western range boundary. Despite the initiation of breeding and release programs the genetic structure and diversity of European common hamster populations is largely unknown. In this study, hamsters from ten localities in five European countries were investigated. Mitochondrial control region was sequenced from 145 animals representing all sampled populations. 385 hamsters were screened for polymorphisms at 11 microsatellite loci. Both marker systems revealed extensive genetic differentiation among European common hamsters. Western populations displayed very low levels of mtDNA diversity (H = 0 - 0.2, Alsace, Limburg, Flanders, Baden-Wuerttemberg) compared to eastern populations from Saxony-Anhalt, Thuringia and Southern Moravia (H = 0.663 – 0.816). Microsatellite analyses revealed a similar pattern with low to moderate diversity values in western hamsters (A = 1.636 - 5.364; H_e = 0.111 – 0.504) and high levels of polymorphism in eastern hamsters (A = 8.909 - 9.818; H_e = 0.712 - 0.786). High microsatellite based *Fst* measures (up to 0.635) suggest a typical island model of distribution with no current gene flow between most areas. Western hamster populations exhibit obvious similarities in mitochondrial haplotype and microsatellite allele distributions. Gene trees group western hamsters consistently together on the same branch but bootstrap values never reached significance.

Das Projekt und die Experimente wurden durch mich konzipiert. Die Proben wurden zum Teil durch die Mitautoren bereitgestellt. Fragmentanalysen und Sequenzierungen wurden unter Mithilfe der technischen Assistentinnen G. Becke, B. Gebhardt und S. Hofmann durchgeführt. Sämtliche Auswertungen der Daten erfolgten durch mich. Das Manuskript wurde von mir geschrieben.

There are strong indications that low diversity in western populations is partially caused by a joint historic founder event and not only by recent population break downs. Overlapping mitochondrial haplotypes prove a close association between western hamsters and animals from the east German range in the recent past which does not support the existence of a separate subspecies *C. c. canescens* in Europe. Hamsters from southern Moravia emerged as the genetically most distinguished population and could be part of a different genetic lineage in Europe.

INTRODUCTION

The common hamster (*Cricetus cricetus*), a formerly characteristic mammal of the open landscape in Europe, experienced a dramatic population decline during recent decades. Western European countries like the Netherlands and France initiated urgent captive breeding and reintroduction programs to prevent the disappearance of their local hamster populations. Other conservation measures support special agricultural cultivation schemes to maintain the species in its accustomed areas and to avoid further population fragmentation.

Hamsters are semi-fossorial rodents constructing their extensive burrow systems preferentially in deep loess and clay rich soils. Under favourable conditions, a female can raise 2 - 3 litters with 4 - 18 offspring in a season and female youngsters may even reproduce in their first year (Petzsch 1950, Vohralik 1974). This high productiveness culminates in occasional outbreaks during so called ″hamster years″. Telemetry and capture-recapture studies have shown that the species is rather sedentary (Weinhold 1996). Only males and juveniles after disintegration occasionally disperse over larger distances (Karaseva 1962). Natural migration barriers are foremost provided by mountainous regions (Vohralik and Andera 1976, Berdyugin and Bolshakov 1998) but there are reports about hamsters crossing extensive river systems such as the Dnestr-Liman during gradation years (Calinescu 1931). Mass appearances seem to provide the key mechanism for long distance dispersal. The geographic range of the common hamster extends from the Yenissey in Siberia into Europe. Its main distribution center lays in the eastern steppe areas whereas hamsters in central and western Europe are restricted to rather isolated areas. Animals in these regions are strongly bound to farmland. This makes the species extremely sensitive to anthropogenic activities and a good model to study the direct human influence on natural mammal populations. The most western range limit proceeds

through the Netherlands, Belgium and France. Common hamsters from there are sometimes considered to form an own subspecies *C. c. canescens* NEHRING, 1899 which is separated from *C. c. cricetus* (LINNAEUS, 1758) in central and eastern Europe (e.g. Mitchell-Jones 1999, but see Niethammer 1982; Grulich 1987).

Adjacent to the western populations, larger hamster areas are found in eastern Germany, the Czech Republic, Slovakia and Hungary. Hamsters were abundant in Europe until 30 - 40 years ago. Local populations in eastern Germany reached densities allowing a commercial harvest of hamster furs. In the late 1960's more than 1 million animals per year were caught in the province of Saxony-Anhalt alone. Such densities are unusual for the species and are attributed to the beneficial agricultural management at the time. During last decades intensified agricultural land utilisation combined with growing infrastructure caused a dramatic decline both in hamster numbers and occupied areas. As a consequence, common hamsters are now protected under the EU habitats and species directive as well as the Bern Convention (Mitchell-Jones 1999).For ongoing protection measures it is essential to gain information about the remaining genetic diversity of common hamster populations. This will not only allow the identification of evolutionary units but also help to assess the self-sustainability of currently threatened populations.

Main aim of this study was to elucidate the genetic structure and variation of western and central European common hamsters from a number of representative populations. A second goal was to investigate historic relationships of today's fragmented hamster sites.

MATERIALS AND METHODS

Population sampling

Common hamsters from ten regions covering five European countries were investigated. All sampling areas were defined as populations considering the low dispersal rates of the species and the extensive urban infrastructure separating most sites. Geographic locations of all sampled populations are shown in Figure 1.

German samples included six different regions. Two populations were sampled in the provinces of Saxony-Anhalt (1994 - 2000) and Thuringia (1993 -1999). A third sample came from an isolated urban area in the city of Goettingen/Lower-Saxony (1999). All three populations are placed in a formerly connected, main distribution area in central Europe. Another population was collected near Heidelberg/Mannheim in the

province of Baden-Wuerttemberg and is placed between the upper Rhine and the river Neckar. The two remaining sites lie in the provinces of Northrhine-Westfalia and Rhineland-Pfalz from which only a few animals were obtained.

French sample consisted of hamsters from the Bas-Rhin area of the Alsace region in north-eastern France. Animals were collected near Strasbourg between 1999 and 2000. Sixteen individuals from 1999 and 2000 represent the remaining Dutch hamster population in the province of Limburg. Fifteen individuals (7 males, 8 females) among them five juveniles were caught at Heer near Maastricht. They form the stock of a breeding and reintroduction program in the Netherlands. To increase the sample size, we included 12 museum skins collected between 1947 and 1980 not more than 40 km away from Heer.

Ten hamsters were sampled between 1998 and 2001 at three different sites within the only existing hamster region along the Flemish/Wallonien boarder west of Brussels. The occurrences of the common hamster in Belgium are considered as highly vulnerable.

Tissues of hamsters from the Czech Republic were taken between 1998 and 1999 near the city of Brno in southern Moravia.

Investigated hamster populations are presumed to represent two genetic lines or subspecies; the western common hamster *C. c. canescens* (Flanders, Limburg, Alsace, Baden-Wuerttemberg, Northrhine-Westfalia and Rhineland-Pfalz) and the eastern common hamster *C. c. cricetus* (Saxony-Anhalt, Thuringia, Lower-Saxony and Southern Moravia).

DNA extraction

Genomic DNA isolation from a variety of fresh or ethanol fixed materials such as ear, liver, muscle, hair or skin followed a standard protocol supplied with the E.Z.N.A. Tissue DNA Kit II system (peqlab Biotechnologie). DNA from museum specimens was extracted using a DNeasy Tissue Kit (Qiagen) in a molecular laboratory in Wageningen under special precautions. All remaining laboratory work was done at the Institute of Zoology at Halle University.

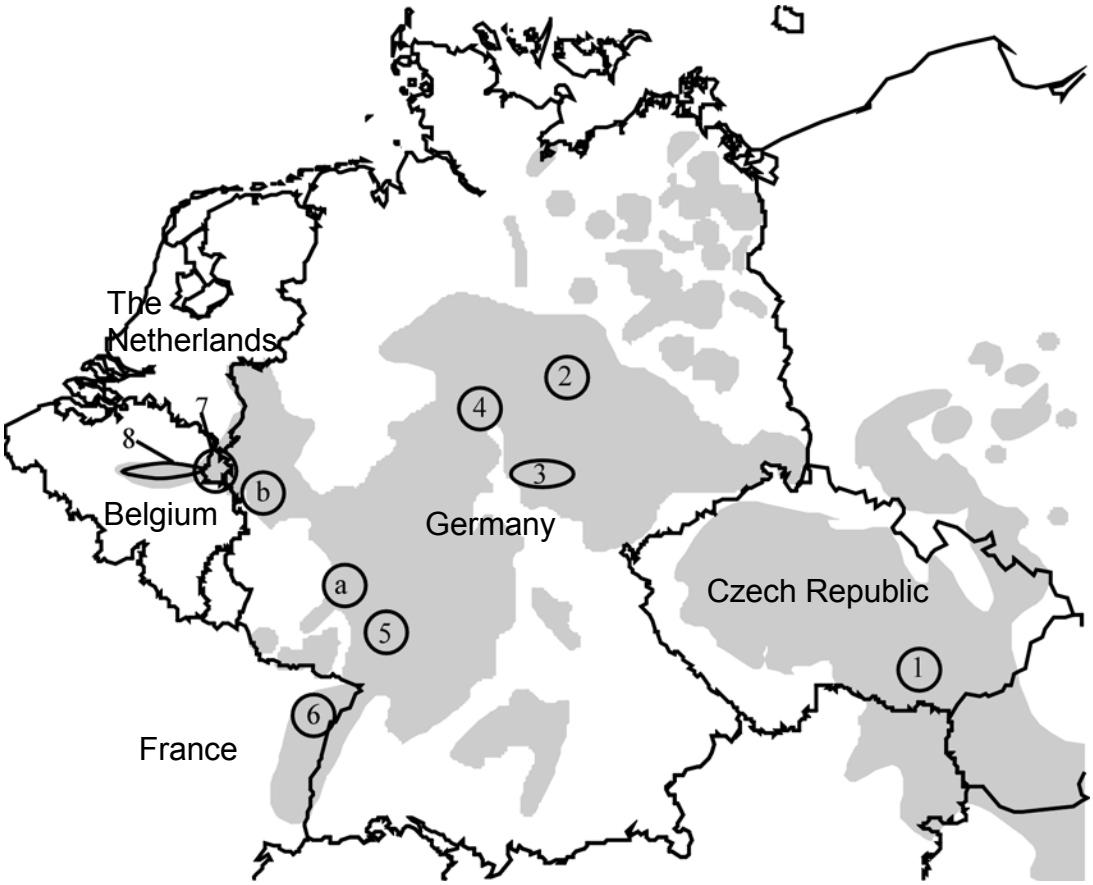

Fig. 1 Distribution of the common hamster in western and central Europe. Circles describe sampling areas: 1 = Southern Moravia, 2 = Saxony-Anhalt, 3 = Thuringia, 4 = Lower Saxony, 5 = Baden-Wuerttemberg, 6 = Alsace, 7 = Limburg, 8 = Flanders, a = Rhineland-Pfalz, b = Northrhine-Westfalia.

D-loop sequence analysis

Partial sequences of the mitochondrial control region were obtained from 145 individuals covering all sampled localities. A maximum of 20 individuals per population was analysed. If an assigned population covered a wider range we sequenced animals from as distant points as possible.

Primers K and B which work in a variety of rodents (Faulkes et al. 1997) were used to amplify a part of the control region including the first variable domain. PCR was performed with 50 ng of genomic DNA, 40 pmol of each primer, and 2 PCR beads (Ready-To-Go system, Amersham) in a 50μl reaction volume. Thermocycling was carried out in an UNO II machine (Biometra). After an initial denaturation step at 94°C for 180 sec., the reaction proceeded for 35 cycles as follows; 60 sec. at 94°C, 60 sec. at 54°C, 120 sec. at 72°C. Amplified products were purified with MicroSpin^{TM} S-300 HR columns (Amersham). Cycle sequencing of double-stranded fragments was carried out with the original, end-labeled (Cy5 amidite) PCR primers in both directions. Reactions were run on an A.L.F.express II automated sequencer (Amersham). 337

bp of DNA sequence were aligned in PROSEQ (v. 2.9, D.A. Filatov, University of Birmingham).

D-loop statistics

Haplotype diversity (*H*) and nucleotide diversity (*Pi*) were calculated in DNASP (Rozas and Rozas 1999). Pairwise *Fst's* were computed using AMOVA in ARLEQUIN v. 2000 (Schneider et al. 2000). To establish phylogenetic relationships a maximum parsimony tree using the close-neighbour-interchange search option was constructed in MEGA 2.1 (Kumar et al. 2001). A published d-loop sequence from the Chinese hamster *Cricetulus griseus* (GenBank D29972, Nakamichi et al 1998) served as an outgroup. One thousand bootstrap replicates were generated to support each node. Additionally, we constructed a haplotype network based on statistical parsimony estimates (Templeton et al. 1992) using the TCS software (Clement et al. 2000). Networks often show better genealogies compared to traditional tree building methods when divergence between haplotypes is low. Pairwise *Fst* estimates were correlated with geographical distances to test for a potential association between genetic and physical distance.

Microsatellite genotyping

Allelic variation at 11 microsatellite loci (Neumann and Jansman submitted), 10 dinucleotides (Ccr*µ*3, 4, 10, 11, 12, 13, 15, 17, 19, 20) and 1 tetranucleotide (Ccr*µ*6), was examined in 358 individuals. PCR was performed using the Ready-To-Go-system (Amersham). Ten pmol of each primer (one either labeled with Cy5 for A.L.F.express II, Amersham or HEX/TET for ABI 377, Perkin Elmer) and 10 - 50 ng of genomic DNA were added to a total volume of 12.5 *µ*l. PCR was performed at 52-54°C annealing temperature as described for d-loop analyses. Alleles exhibiting unusual length differences were cloned into the pGEM-T-vector system (Promega) and subsequently sequenced using the T7 sequencing kit (Amersham).

Microsatellite data analysis

Statistics was carried out with eight populations. The number of specimens collected in Northrhine-Westfalia (*n* = 6) and Rhineland-Pfalz (*n* = 2) was low and their data were kept out from most quantitative analyses.

Genetic variation was measured as the mean number of alleles (*A*), observed (H_O) and expected heterozygosities (H_e; Nei 1973). Tests for Hardy-Weinberg equilibrium (HWE) per locus and per population were performed by the Markov chain method (Guo and Thompson 1992) using GENEPOP (Raymont and Rousset 1995).

Population differentiation was based on genotypic and genic variation as implemented in the same program. FSTAT (Goudet 1995) was used for the computation of pairwise *Fst*-values to quantify the degree of isolation between sites. Individuals from populations with low sample size were tested for genetic similarities to larger samples via an assignment test (WHICHRUN, Banks and Eichert 1999). The program calculates a maximum likelihood ratio L(n)/L(max) to allocate individuals to their most probable source population. The program BOTTLENECK (Cornuet and Luitkart 1996) was used to test for recent bottlenecks affecting the expected/observed heterozygosity ratio. Inbreeding coefficient *Fe* was estimated as $Fe = 1 - (H_{island}/H_{mainland})$ (see Laikre and Ryman 1991) where $H_{mainland}$ refers to expected heterozygosities of the most polymorphic populations.

To assess historic relationships between populations we identified alleles limited to a small number of populations. A maximum parsimony tree was constructed based on Nei's standard genetic distance *Ds* (Nei 1972) with POPULATIONS. Bootstrapping was carried out over the number of loci. The resulting tree was drawn in TREEVIEW (Page 1996).

Mantel's test (Mantel 1967) was performed to assess the correlation between geographic location and calculated distance measures (MANTEL v. 2.0, A. Liedloff, Queensland University, Brisbane, Australia). Geographical distances were measured between the centers of sampling areas using ENCARTA (Microsoft).

RESULTS

D-loop analyses

Control region diversity in the common hamster was found to be lower than in other murine species e.g. striped field mouse *Apodemus agrarius* (Koh et al. 2000) but was in the range of lemming populations *Dicrostonyx groenlandicus* (Ehrich et al. 2000, 2001). Obtained partial d-loop sequences show about 86% homology (including two gaps) to the only other published hamster sequence derived from *Cricetulus griseus* (Nakamichi et al 1998). This high sequence homology serves as an indication that we analysed true mitochondrial sequences and no nuclear pseudogenes.

Table 1 Genetic diversity (*H*) and nucleotide diversity (*Pi*) of common hamster populations based on individual d-loop haplotypes.

Site	*N*	No. of haplotypes	Haplotypes (*f*)	*H* (SE)	*Pi* (SE) in %
Saxony Anhalt	20	5	dl01(0.55); dl02(0.2); dl03(0.15); dl04(0.05); dl05(0.05)	0.663 (0.021)	0.4 (0.02)
Thuringia	20	7	dl01(0.1); dl02(0.05); dl06(0.25); dl07(0.05); dl08(0.05); dl09(0.15); dl10(0.35)	0.816 (0.013)	0.7 (0.01)
Lower Saxony	12	2	dl04(0.083); dl11(0.917)	0.167 (0.039)	0.3 (0.03)
Baden-Wuerttemberg	20	2	dl1(0.95), dl12(0.05)	0.100 (0.020)	0.03 (0.01)
Alsace	20	2	dl01(0.9); dl13(0.1)	0.189 (0.024)	0.06 (0.01)
Limburg	20*	1	dl01(1.0)	-	-
Flanders	10	2	dl01(0.9); dl14(0.1)	0.200 (0.049)	0.06 (0.01)
Southern Moravia	20	5	dl15(0.35); dl16(0.3); dl17(0.1); dl18(0.2); dl19(0.05)	0.774 (0.012)	0.36 (0.01)
Northrhine-Westf.	2	-	dl01	-	-
Rhineland-Pfalz	1	-	dl01	-	-
Average Europe	*145*	*3.3*	*dl01(0.566); dl02(0.034); dl03(0.021); dl04(0.014); dl05(0.007); dl06(0.034); dl07(0.007); dl08(0.007); dl09(0.021); dl10(0.048); dl11(0.076); dl12(0.007); dl13(0.014); dl14(0.007); dl15(0.048); dl16(0.041); dl17(0.014); dl18(0.028); dl19(0.007)*	*0.364*	*0.24*

* For the Dutch hamster population we analysed 10 recent hamsters and 10 historical samples.

D-loop haplotype frequencies and diversity indices are presented in Table 1. Nineteen haplotypes (CCRdl01-CCRdl19) were identified in 145 hamsters. Sequences were deposited in EMBL (AJ550189 - AJ550207). Seventeen sites proved variable among 337 bp scored. All mutational changes represent transitions. Seven substitutions comprise singletons and 10 were informative under parsimony. Sequence divergence between all haplotypes ranged from 0.3% to 2.4%. Average within population divergence of haplotypes measured less than 1% with the exception of Lower-Saxony (1.78%). Haplotype numbers (1 - 7) and frequencies (0.05 - 1.0) varied extraordinarily across populations. The highest number of haplotypes was found in

Thuringia (7), Saxony-Anhalt (5) and Southern Moravia (5). Gene diversity *H* ranged from 0.663 - 0.816 in these populations. Their nucleotide diversity *Pi* varied between 0.36% - 0.7% and maximum haplotype frequency measured 55% (CCRdl01 in Saxony-Anhalt). All remaining populations revealed a much lower variability. Lower-Saxony which is geographically close to Saxony-Anhalt and Thuringia is almost fixed for a unique haplotype (CCRdl11, 0.917%). A single individual of the same population revealed a second haplotype differing at six positions from the other. This results in an unusual high *Pi*-value (0.3%) compared to the observed low haplotype diversity (*H* = 0.167). Reduced levels of polymorphism (*H* = 0.1 - 0.2, *Pi* = 0.03 - 0.06%) appear to be a general feature of all western populations. Limburg is fixed for a single haplotype and only two dloop variants are present in Baden-Wuerttemberg, Alsace and Flanders. All four population share a predominant haplotype CCRdl01 (≥ 0.9%) which is also found in Thuringia and Saxony-Anhalt. Baden-Wuerttemberg, Alsace and Flanders exhibit additional rare haplotypes which differ in all three cases by a single transition from the common type resulting in very low *Pi*-values (0.03 - 0.06%).

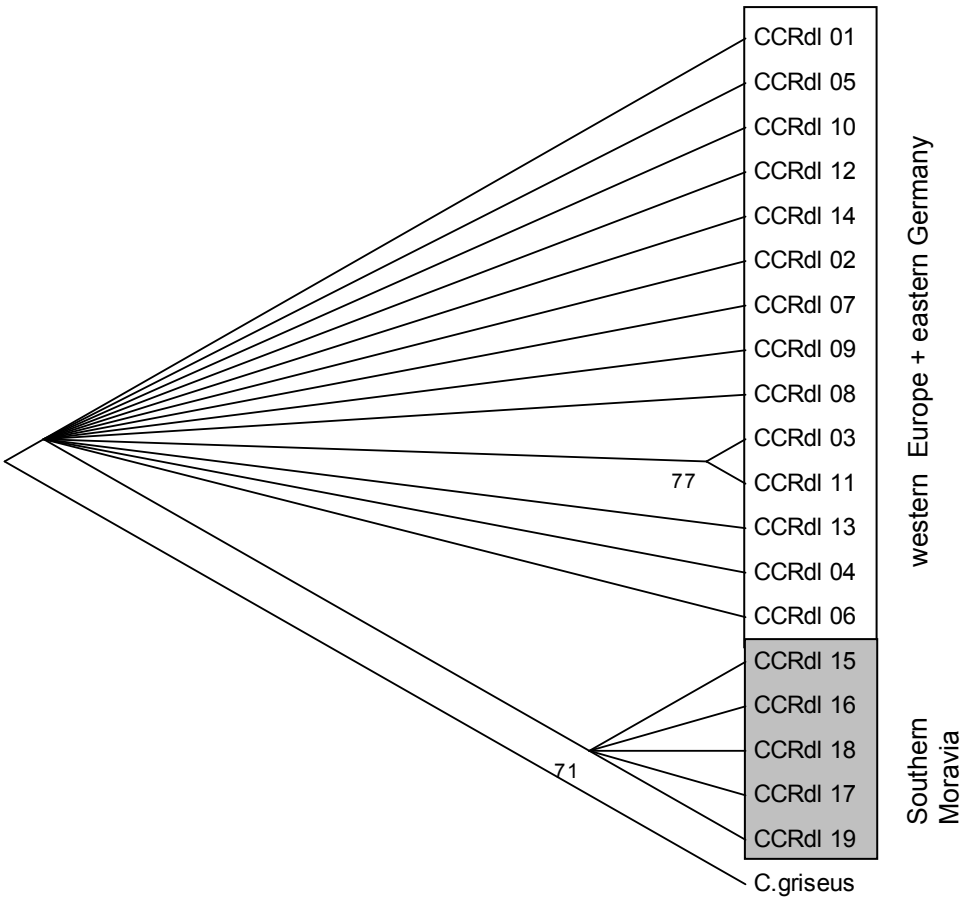

Fig. 2 Maximum parsimony tree of d-loop haplotypes from 10 different common hamster populations (close-neighbour-interchange search method, 1000 bootstrap replicates, drawn in *MEGA*).

Maximum parsimony tree (Figure 2) and a statistical parsimony cladogram (Figure 3) identify only hamsters from Southern Moravia as a separate unit. However, bootstrap support for that is low although, both clusters do not share any haplotypes. Pairwise *Fst*'s support a differentiation of Southern Moravia (0.729 – 0.896, p<0.0001) from other hamsters. Only comparisons with Lower Saxony reach similar high *Fst*'s (0.641 – 0.901, p<0.0001). Remaining populations show low to moderate differentiation (0 – 0.299) with often insignificant p-values after correction for multiple tests. A comparison of mitochondrial based pairwise *Fst*'s and geographic locations produced a correlation between genetic and physical distance in European hamster populations. The calculated correlation is most likely an artifact because it results from the combination of two point clusters with different pairwise relationships.

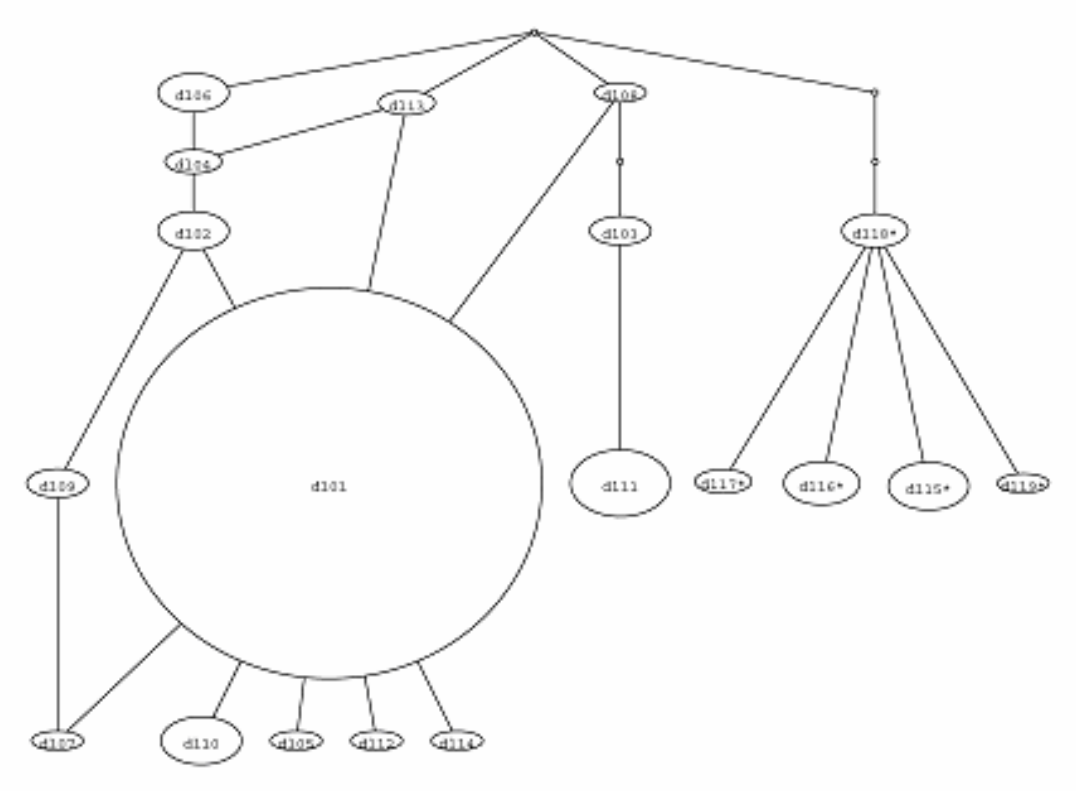

Fig. 3 Statistical parsimony network showing the relationship of d-loop haplotypes in European common hamsters. Sizes of circles correlate with the frequency of haplotypes in the entire sample. Small circles represent missing haplotypes. Asterisks mark haplotypes found in Southern Moravia. The original network was edited to remove all crossings.

Microsatellite variability

Microsatellite allele diversity (Table 2) varied extremely across sampling areas. The three largest populations from Saxony-Anhalt, Thuringia and southern Moravia proved most variable with mean observed and expected heterozygosities between 0.731 - 0.772 and 0.712 - 0.786, respectively. Allele numbers range from 8.909 -

9.818. Western populations showed lower diversity values H_e = 0.111 – 0.504 and A = 1.636 - 5.364. All these populations contained either monomorphic loci (Limburg: 3 loci, Flanders: 7 loci) or loci of very low variance (Alsace: Ho = 0.029 at Ccr*μ*13). Corresponding to that, we calculated high levels of inbreeding in western hamsters (0.338 - 0.854) relative to their conspecifics further east. Combined mean allele numbers between “high” and “low” polymorphic populations differed significantly (Wilcoxon signed rank test, two tailed $p < 0.003$). Tests of recent population bottlenecks could only be performed with reservations. The number of tested microsatellites falls short of the recommended 20 and is even more reduced in western populations which exhibit monomorphic loci. Testing of the most polymorphic hamsters from Southern Moravia, Saxony-Anhalt and Thuringia revealed evidence for recent bottlenecks due to a significant excess of heterozygotes ($p<0.005$, one tailed Wilcoxon test) in all three populations assuming infinite allele mutation-drift equilibrium (IAM). No such bias was detected according to a stepwise mutation approach (SMM). Significant deviations from Hardy-Weinberg expectations after sequential Bonferroni correction were found at Ccr*μ*3 in Saxony-Anhalt and at Ccr*μ*11, Ccr*μ*19 and Ccr*μ*6 in the Limburg population. One explanation therefore could be inconsistent length variation at locus Ccr*μ*3 in the population from Saxony-Anhalt leading to scoring difficulties in heterozygotes. The high proportion of loci causing HWE disequilibrium in Limburg can be attributed to the heterogeneity of the sample containing additional museum specimens. Allele and genotype distributions differed highly significant ($p<0.001$) between all populations. An overview on the distribution of alleles is given in Figure 4. Pairwise *Fst*-values predict high degrees of isolation between sample sites. Smallest distances were found between Saxony-Anhalt, Thuringia and southern Moravia (SAnh/Thur = 0.085 – SAnh/SMor = 0.148) whereas all other population comparisons range from 0.166 – 0.635. A population tree based on *Nei’s* standard distance *Ds* (Figure 5) branches western hamsters separately but with low bootstrap support. A potential split between Southern Moravia and the east German populations from Saxony-Anhalt and Thuringia gained also low support. An assignment test identifies hamsters from Rhineland-Pfalz and Northrhine-Westfalia as parts of the western group. The probability that they belong to one of the western populations was 100 – 10,000 x higher than being part of an eastern population. Private alleles of diagnostic value for the establishment of past population relations were found at loci Ccr*μ*3 and Ccr*μ*6.

A common allele at locus Ccrμ3 present in three different populations Saxony-Anhalt (*f* = 0.634), Thuringia (*f* = 0.286) and Lower Saxony (*f* = 1.000) shows an additional A-insertion upstream of the most variable CA-repeat motif. Sequencing of the allele revealed another three base changes inside the CA cluster leaving the repetition frame unaltered. Ccrμ6, a tetranucleotide, proved highly variable with a very complex structure. The fragment pattern consists of two groups of alleles divided by often more than a 100 base pairs. The number of small sized alleles was much lower (n = 7) compared two large alleles (n = 28) which may attribute to different mutation rates. Small alleles harbour between 9 and 15 GAAA repeats. Their sequences were identical in all populations present (Southern Moravia, Saxony-Anhalt, Thuringia, Lower Saxony). In contrast the satellite sequence in large alleles is characterized by a number of substitutions interrupting pure GAAA chains. A GAGAGG insertion in the middle of the repeat region is of diagnostic value. This motif is characteristic for all but specimens from Southern Moravia. Large alleles in this population are characterized by an unique length change due to the insertion of a third GA. No correlation between geographical distance and genetic distance *Fst* could be established.

DISCUSSION

Genetic variability and population history of western common hamster populations

The analysis of partial D-loop sequences revealed significantly reduced levels of genetic diversity in western hamsters compared to populations from southern Moravia (Czech Republic), Saxony-Anhalt and Thuringia (eastern Germany). At the first sight the result is not surprising as varying levels of diversity could be correlated with different population sizes of eastern and western hamsters. Otherwise, in the light of contemporary population collapses in western countries a loss of genetic variation is inevitable. A most severe breakdown was observed in Dutch populations which went down to a few tens of individuals until present. Therefore, one explanation for the observed loss of genetic variability in western hamsters is a combination of low population size and large density fluctuations.

Small populations are subject to increased genetic drift and more intense bottleneck effects in particular when these populations meet sub-optimal environmental conditions along distribution boundaries. This should lead to a random accumulation of different haplotypes in different populations. An example for a drift effect was seen in

Lower Saxony. The investigated sample revealed almost fixation for haplotype CCRdl11 not detected in neighbouring populations from Saxony-Anhalt and Thuringia. Hamsters from this site provide a good example how human infrastructure creates an island population by interrupting gene flow from adjacent hamster areas. However, such regional events do not sufficiently explain similar haplotype distributions in western hamsters. At first, the analysis of ten museum specimens collected between 1947 and 1980 at various places in Limburg showed that all of them share the same haplotype CCRdl01 with recently sampled Dutch individuals.

Table 2 Genetic variability of common hamster populations evaluated with 11 microsatellite loci. Allele numbers (A), observed (*Ho)* and expected (*He)* heterozygosities are given as means with their standard errors. Inbreeding coefficient F_e was calculated against the mean of the *He* values derived from the three most outbred populations. Data from Northrhine-Westfalia (n=6) and Rhineland-Pfalz (n=2) are not included because of too small sample size.

Site	N	Mean *A*	Mean H_o	Mean H_e	F_e
Saxony-Anhalt	97	8.909 (0.948)	0.731 (0.034)	0.712 (0.045)	-
Thuringia	35	9.545 (1.275)	0.750 (0.037)	0.786 (0.034)	-
Lower Saxony	16	4.091 (0.456)	0.415 (0.096)	0.404 (0.092)	0.469
Baden-Wuerttemberg	32	4.091 (0.456)	0.385 (0.067)	0.419 (0.066)	0.449
Alsace	67	5.364 (0.834)	0.502 (0.079)	0.504 (0.076)	0.338
Limburg	28	3.182 (0.711)	0.201 (0.050)	0.325 (0.078)	0.573
Flanders	10	1.636 (0.364)	0.105 (0.067)	0.111 (0.073)	0.854
Southern Moravia	65	9.818 (1.007)	0.772 (0.018)	0.784 (0.0023)	-
Average Europe	*350*	*5.83*	*0.48*	*0.51*	-

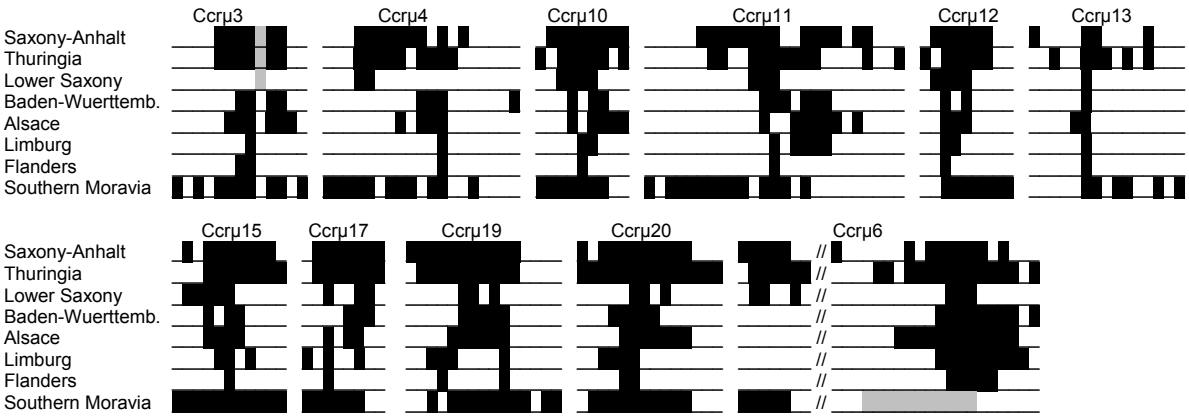

Fig. 4 Allele size distributions at 11 microsatellite loci in the common hamster. Black bars refer to present and empty bars to missing allelic states. Grey bars indicate alleles of unusual size. (//) marks the gap between small and large alleles at locus Ccr*μ*6.

This finding is surprising because some of the specimen were collected prior of the rapid population drop in the Netherlands. Second, CCRdl01 appeared to be the predominant haplotype in all other western hamsters from Baden-Wuerttemberg, Alsace and Flanders. CCRdl01 was also found in three samples from Northrhine-Westfalia and Rhineland-Pfalz. Only a few rare haplotypes exist in Baden-Wuerttemberg, Alsace and Flanders. Statistical parsimony network analysis and a maximum parsimony based gene tree demonstrate star-like distribution of haplotypes in western hamsters which is best explained by population expansion after a historic bottleneck. CCRdl01 remained in the founding group of individuals from which all other haplotypes evolved due to single transitions resulting in low sequence diversity.

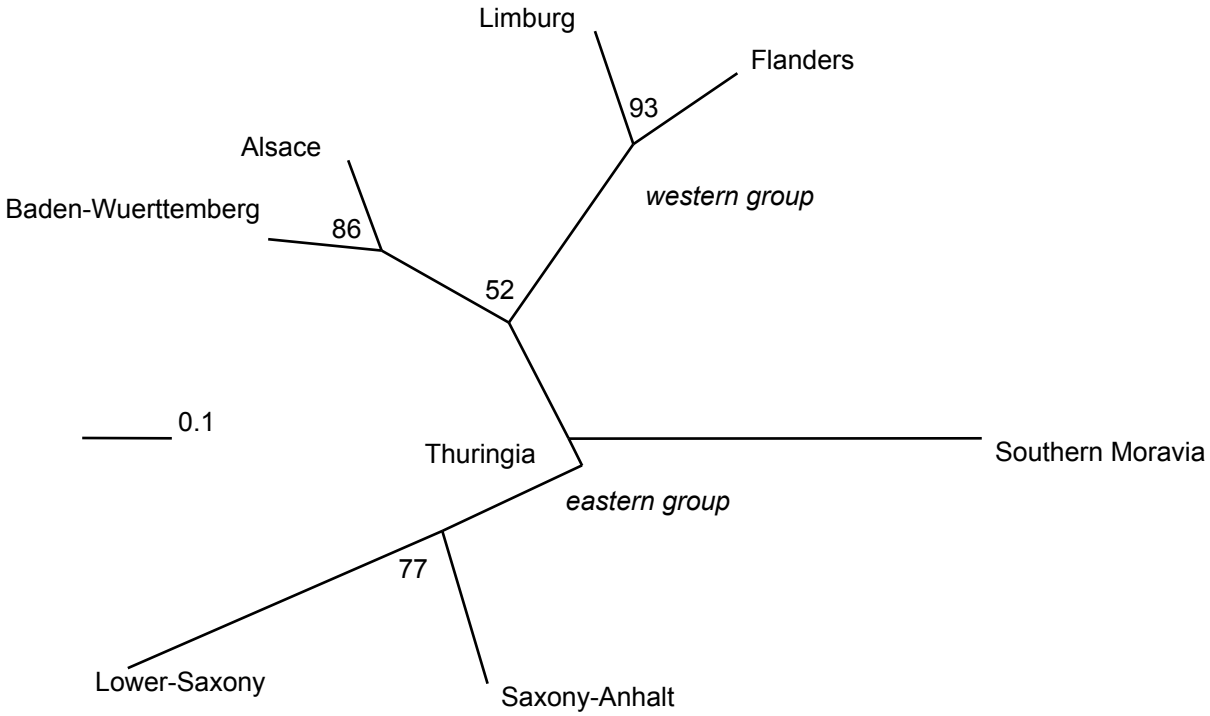

Fig. 5 Neighbour-joining tree of 8 common hamster populations based on allele distributions at 11 microsatellite loci using Nei's standard genetic distance *Ds*. Percentage of support in 1000 bootstrap replicates for each node is shown when it exceeds 50%.

This bottleneck has most likely occurred after the last glaciation within the last 10,000 years when hamsters advanced from their eastern refuge into central and western Europe (Grulich 1987). Post-glacial founder effects have already been discussed as a reason for reduced genetic variation on a geographical scale in other European mammals e.g. the root vole *Microtus oeconomus* (Leijs et al. 1998) or mustelids (e.g. Effenberger and Suchentrunk 1999). Past and present Scandinavian samples of the wolverine *Gulo gulo* (Walker et al. 2001) were monomorphic for a single d-loop haplotype comparable to the situation of western common hamsters. It can be specu-

lated that western hamsters formed the leading edge of dispersing hamsters founding a population at the western side of the Rhine. Whether the Rhine crossing itself or long distance dispersal of a small number of individuals (Hewitt 1996) caused the historic founder event remains unclear. In any case, as a result all western populations should show the same dominating haplotype. When common hamsters retracted to the east due to unfavourable conditions caused by a cooler climate or anthropogenic influence a few remnant populations were left behind shaping today's distribution. Another argument for such a scenario comes from the fact that eastern German hamsters show the same dominant western haplotype in moderate frequencies. This argues strongly for a historic connection between eastern and western hamsters because ongoing gene flow can be excluded due to the geographic distances and barriers provided by human infrastructure. Hamster populations in eastern Germany may be the remains of the past source population from which hamster expanded to the west. Variation at microsatellite loci largely confirms mitochondrial data. Microsatellite diversity in the most polymorphic populations from Saxony-Anhalt, Thuringia and Southern Moravia is comparable to values in other wild rodent species such as the bank vole *Clethrionomys glareolus* (Gerlach and Musolf 2000) or the Mongolian gerbil *Meriones unguiculatus* (Neumann et al. 2001). It is difficult to decide whether recent declines observed in these populations had an impact on genetic variability but if so, the effects seem to be minor. However, there are indications for present genetic bottlenecks in all three outbred populations ($p<0.005$, Wilcoxon test). The finding is in agreement with observed census size fluctuations e.g. in the population from Saxony-Anhalt where burrow densities in spring changed from 2.3/ha - 0.5 /ha during sampling years. A completely different situation is found for western hamsters. Allelic diversity in these populations reached only low (Belgium; H_e = 0.111) to moderate values (Alsace; H_e = 0.504) confirming that narrow gene pools are a general characteristic of western hamster populations. Proving that western hamsters passed through an ancestral bottleneck using allele frequency data turned out to be difficult. Reason therefore is the dynamic population structure of the species. Isolation plus recurrent size fluctuations change microsatellite allele distributions in a random fashion and erase traces of a common genetic origin rather quickly. High numbers of invariant loci in Belgium and Limburg are therefore more likely the result of recent population drops. A conclusion which is supported by a previous comparison of recent and historic samples from the Dutch population using the same set of

microsatellites (Neumann and Jansmann submitted). Moderate diversity in Alsace shows that western hamsters managed to retain some microsatellite variability after the predicted founder event. A similar effect was described from other mammalian founder events which were followed by a rapid population expansion e.g. the European rabbit *Oryctolagus cuniculus* in Australia (Zenger et al. 2003). Nevertheless, there is evidence for common ancestry of western hamsters from microsatellite data. Western hamsters proved almost monomorphic for the same allele at locus Ccr*µ*13 (Flanders, Limburger, Baden-Wuerttemberg: *f* = 1, Alsace: f = 0.97) but this particular allele went also to fixation in Lower Saxony. Unusual high shared allele frequencies were observed at two further Loci; Ccr*µ*3 (west: 0.61-1.0/east: 0-0.24) and Ccr*µ*15 (west: 0.72-1.0/east: 0.01-0.47). None of the western hamsters inherited the atypical sized allele at locus Ccr*µ*3 which is typical for Lower Saxony, Thurinigia and Saxony-Anhalt. The characteristic gap dividing low and large sized alleles at locus Ccr*µ*6 was found in all eastern population but not in the west. Western hamsters exhibit exclusively large alleles. The genetic similarity of western hamsters is also illustrated by a neighbour-joining tree branching western hamsters together even bootstrap values are below significance. Finally it can be concluded that both marker systems provide evidence that the genetic erosion in western hamsters started already with an ancestral founder event during the plost-glacial recolonization event of the Rhine valley. Forthcoming negative population size fluctuations were more likely responsible for the currently observed high degree of microsatellite monomorphism in Belgium and the Netherlands.

"Island" structure and isolation by distance

Western hamster populations show a high degree of isolation. High microsatellite *Fst's* ranging up to 0.635 predict a complete absence of gene flow in recent populations. The finding confirms the "island" character of today's western relict sites due to anthropogenic habitat fragmentation. In our case microsatellite based *Fst* values are better suited to estimate the extent of population isolation because most western hamsters share the same mitochondrial haplotype. The dominance of a single haplotype in western hamsters is, as already discussed, more likely the consequence of a historic bottleneck and does not account for extensive gene flow. That recurrent and historical events can have confounding effects on genetic distance measures has already been shown in other studies (Gerber and Templeton 1996, Charlesworth

1998). Despite genetic differences between western sites there is an obvious clustering of nearby regions. Hamsters from Flanders, Limburg and Northrhine-Westfalia (according to association tests) confine to the same geographical region, a former hamster area which extended eastwards to the German Rhine area around Cologne (see Niethammer and Krapp 1982 and map provided). A progressing disintegration of this formerly connected hamster region becomes evident considering the number of monomorphic microsatellite loci within the three hamster samples; Flanders (7), Limburg (3), Northrhine-Westfalia (1, but low sample size). A similar situation is found for Alsace and Baden-Wuerttemberg two geographically close populations which are divided by the Rhine river. It seems that at least the upper Rhine formed not an effective impediment for gene flow in the past. Pairwise microsatellite *Fst* values between Southern Moravia, Saxony-Anhalt and Thuringia measured considerably lower than in western hamsters 0.089 - 0.149. If this accounts for true gene flow or is the result of recent common origin cannot be decided. At least Saxony-Anhalt and Thuringia have formed a largely connected hamster area in the past. More problematic is the interpretation of the relatively low microsatellite *Fst* measures in comparisons with Southern Moravia. The finding is in opposition to the exclusively private mitochondrial haplotypes in Czech hamsters and corresponding high mitochondrial *Fst's*. Unique large alleles at microsatellite locus Ccrμ6 in Southern Moravia argue against male mediated gene flow. The observed controversy can be explained either by allele size homoplasy due to the fast mutation rate of microsatellites (Estoup *et al*. 1995, 2002) or different effective population sizes for the two marker systems. No significant correlation between genetic distance and linear geographic distance could be established using microsatellite or mitochondrial based *Fst* values. A potential association found with mitochondrial data is most likely an artifact by combining a group with large differentiation values (all combinations with Southern Moravia and Lower Saxony) and a second group with lower differentiation (all others). Both population clusters itself show no correlation with physical distance. The lack of correlation could be explained by the poor dispersal abilities of the species and the absence of high density periods triggering larger distance migration. Human-constructed barriers that obstruct dispersal are another likely reason for that. Common hamsters represent another example how anthropogenic land use enhances the fragmentation of small mammal populations. A problem which applies to a variety of other rodents (Barrett et al. 1999, Oli et al. 2001, Tallmon et al. 2002).

Is there more than one common hamster subspecies in Europe?

Our study revealed a surprising degree of genetic structuring in European common hamster populations regarding that the species may have recolonized Europe not before the end of the last glacial period (Grulich 1987). Most populations are mainly distinguished by frequency changes of microsatellite alleles and d-loop haplotypes showing that lineage sorting played a much greater role for differentiation than accumulation of new mutations. The almost entirely quantitative nature of differences found between western hamsters *C. c. canescens* and hamsters from eastern Germany which belong to the nominate form *C*. *c*. *cricetus* leaves some doubt on currently upheld views on sub-speciation in Europe (Mitchell-Jones1999, Spitzenberger 2001). Haplotype network and gene trees associate western hamsters consistently with populations from eastern Germany. However, the presented genetic data are still insufficient to exclude the existence of two separate subspecies in Europe. More intriguing is that the most distinguished population comes from Southern Moravia. Czech hamsters show an allele length change at locus Ccr*µ*6 which is not found in any other investigated hamster population. Another feature is the existence of exclusively unique d-loop variants with the highest degree of sequence divergence of 1.2 – 2.4% compared to all other recorded haplotypes. Phylogenetic trees and statistical parsimony network analyses also identify Southern Moravia as a distinct cluster, but bootstrapping does not support the significance of the split. Nevertheless, there is evidence for genetic isolation of these hamsters which is particularily interesting because hamsters from Southern Moravia are considered to belong to the same subspecies as hamsters from Thuringia and Saxony-Anhalt.

Implications for conservation

The obtained results have implications for running captive breeding programs as well as for the management of wild hamster populations in central and western Europe. Genetic variability of most endangered populations in Belgium and the Netherlands is extremely reduced and even below other western hamster populations. It remains to be seen whether the reintroduction program in the Netherlands will succeed because breeding with the current source population comprises another substantial bottleneck for the new population to start with. A positive example for the foundation of a prospering rodent population from a low polymorphic stock was provided by the release of beavers in Sweden (Ellegren et al. 1993). On the other hand the persistence of the

common hamster at its western boundary over perhaps several thousand years could have lead to the accumulation of adaptive genes. Introduction of hamsters form eastern populations may therefore prove disadvantagous. The creation of migration corridors between existing populations would provide a prospective alternative. The observed spatial structure of common hamsters in western Europe raises a general issue for conservationists, the management of isolated populations in sub optimal habitats. The lasting impact of a historic bottleneck implies that western hamsters may have never reached sufficiently high numbers to regain polymorphisms until present. A major reason for that could be an unstable structure due to unfavourable environmental conditions. It might be speculated that present rapid collapses of western hamsters may represent the climax of an ongoing retreat from an advanced position. Moreover, it indicates an even greater dependency of these populations hamster on a large scale “hamster friendly” agricultural management to prevent extinction. Another problem arises for the management of Evolutionary Significant Units (Moritz 1994). Until now, conservation measures in Europe are guided by the assumption that only two distinct genetic lines of western and eastern hamsters exist. In contrast, our data point towards a more complex structure of the common hamster. In particular genetic relations between eastern German populations and hamsters from the Czech Republic and Hungary require a detailed reassessment in the future.

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3.4. Die Phylogeografie des Feldhamsters in Europa

Die Phylogeografie beschäftigt sich mit der Verteilung von genetischen Linien im Zusammenhang mit geografischen Gegebenheiten. Sie ermöglicht Rückschlüsse auf historische Prozesse, die die gegenwärtige räumliche Struktur von Organismen beeinflusst haben und bildet damit eine Synthese aus Populationsgenetik, Ökologie, Geografie, Klimatologie und Paläontologie.

Studien zur Genese von Verbreitungsmustern von Pflanzen und Tieren sind heute wesentlicher Bestandteil biologischer Forschung (Avise 2000). Sie liefern nicht nur grundlegende Erkenntnisse zu Mechanismen der Populationsdifferenzierung und Artbildung, sondern zeigen darüber hinaus wie Organismengruppen auf klimatische Veränderungen reagieren. In den gemäßigten Zonen waren es insbesondere quartäre Klimaschwankungen (Wechsel von Wärme- und Kälteperioden), die signifikante Aralverschiebungen von Populationen verursachten (Hewitt 1996, 2004; Taberlet et al. 1998). Intra- und interspezifische Diversität gehen häufig auf Isolation in unterschiedlichen Glazialrefugien zurück. Bedeutende glaziale Rückzugsgebiete lagen im Süden der iberischen Halbinsel, Italien und dem Balkan oder dem kaspischen Raum (Hewitt 1999, 2000; Taberlet et al. 1998; Tzedakis et al. 2002). Allerdings gibt es Einwände gegen ein solches, rein allopatrisches Modell der Populationsdifferenzierung und Speziation. Stewart (2003) empfiehlt ein komplexeres Szenario, welches para- und sympatrische Prozesse beinhaltet, die eine Hybridisierung von Linien während der Wiederbesiedlung von Habitaten verhindert. Neben der Dauer der Isolation hatte auch die Form der Wiederbesiedelung von Arealen wesentlichen Einfluss auf die genetische Differenzierung von Populationen. Das langsame, phalanxartige Vorrücken in neue Habitate hinterließ homogenere Strukturen als das schnelle Voraneilen von Pionierpopulationen (Nichols and Hewitt 1994; Ibrahim et al. 1996). Insbesondere Pionierpopulationen unterlagen starken Bestandsschwankungen (Driftwirkung) und erhöhtem Anpassungsdruck in einer neuen ökologischen Umwelt. Eine Reihe von europäischen Studien untersuchte die Phylogeografie von Kleinsäugern, da deren geringeres Dispersionspotential und kurze Generationenfolge zu einer schnellen Populationsdifferenzierung führt. Dabei richtete man den Fokus vor allem auf Arten mit einer relativ weiten und auch nördlichen Verbreitung (Jaarola und Tegelström 1995; Jaarola und Searle 2002; Brunhoff et al. 2003; Runck and Cook 2005). Dabei wurde gezeigt, dass sich zwar einige Grundmuster der spät- oder post-

glazialen Ausbreitung in Europa wiederholen, wie z.B. die Emigration aus südwest- und südosteuropäischen Refugien (Hewitt 1996; Taberlet et al. 1998), aber auch wesentliche Unterschiede zwischen den Arten zu finden sind (Haynes et al. 2003; Michaux et al. 2003). So überstanden einige Kleinsäuger-Arten die Kälteperioden in Refugien im zentralen Europa, (Bilton et al. 1998; Stewart and Lister 2001; Brunhoff et al. 2003; Deffontaine et al. 2005; Michaux et al. 2005), was teilweise durch Fossilienfunde, die auf das letzte glaziale Maximum datiert wurden, bestätigt wird (e.g. Jánossy 1986; Nadachowski et al. 2003). Die überwiegende Anzahl von phylogeografischen Studien in Europa beschäftigt sich mit Arten (u.a. *Apodemus flavicollis*, *A. sylvaticus*, *Clethrionomys glareolus*) die Waldbiotope bevorzugen bzw. ein breiteres Habitatspektrum bewohnen (Michaux et al. 2003, 2004; Berthier 2005). Hier waren es vor allem Kälteperioden, die einen Rückgang der Wälder bewirkten und damit Populationen in unterschiedliche refugiale Bereiche abdrängten. Europäische Offenland- oder Steppenarten, die ihre Verbreitungsschwerpunkte außerhalb von Mitteleuropa besitzen, sind dagegen kaum untersucht worden. Eine Ausnahme bildet die Feldmaus *Microtus arvalis* mit hoch differenzierten Linien in Osteuropa (Haynes et al. 2003, Heckel et al. 2005). Die östliche Form wird als eigene Art *M. obscurus* angesehen. Kontinentale Formen wie Steppenbirkenmaus *Sicista subtilis* und Feldhamster *Cricetus cricetus* stellen Relikte der weitflächigen Versteppungen Europas dar, die vor allem während kühlerer Abschnitte des Pleistozäns stattfanden (Kahlke 1981). Solche Arten sollten andere räumliche und zeitliche Ausbreitungsmuster zeigen. Eine Untersuchung der Populationsstruktur des Feldhamsters unter phylogeografischen Gesichtspunkten ist deswegen sinnvoll, da die Art an ihrer mitteleuropäischen Verbreitungsgrenze besonders sensitiv auf ökologische und damit Klimaveränderungen reagiert. Ein Verständnis der Populationsgeschichte des Feldhamsters ist wesentlich um die sich fortsetzenden Bestandsrückgänge der Art in weiten Teilen Europas zu bewerten. Bisher wurden vor allem Veränderungen in der landwirtschaftlichen Bewirtschaftung als alleinige Schlüsselfaktoren für den Rückgang des Feldhamsters verantwortlich gemacht. Zum anderen ermöglicht die Identifizierung wichtiger evolutionärer Linien bzw. distinkter Populationen, eine effektivere Koordinierung von Schutzmaßnahmen, um die genetische Variabilität einer Art zu bewahren. Dabei sollen entsprechend dem “evolutionary significant unit“ (ESU)-Konzept stark differenzierte Populationen nicht vermischt werden (Moritz 1995). Dies kann mitunter zu Auszuchtdepression führen (Lynch 1991) z.B. durch den Verlust lokaler Adaptationen

oder das Aufbrechen co-adaptierter Genkomplexe. Crandall et al. (2000) schlug alternativ zum ESU die Festlegung von “managemant units“ vor. Die Eigenständigkeit von Populationen erschließt sich dabei über den Grad von rezentem und historischem Genfluss.

Ausgehend von diesen Überlegungen wurde eine umfangreiche Studie zur Phylogeografie des Feldhamsters begonnen. Ergebnisse zur Verbreitungsgeschichte in West- und Mitteleuropa wurden in der **Publikation: „IV Genetic spatial structure of European common hamsters (*Cricetus cricetus*) – a result of repeated range expansion and demographic bottlenecks“** veröffentlicht. In dieser Studie wurden die genetischen Daten von drei mitochondrialen partiellen Genen (Kontrollregion, Cytochrom b, 16SrRNA) sowie 11 nukleären Mikrosatelliten verarbeitet. Bei den Mikrosatelliten wurden nicht nur Längenpolymorphismen untersucht, sondern auch die Sequenzinformation ausgewählter Loci mit einbezogen. Es konnte gezeigt werden, dass im mittleren und westlichen Europa im Wesentlichen zwei Gruppen von Matrilinien existieren. Eine südliche, pannonische Feldhamsterlinie besiedelt das Karpatenbecken, während eine nördliche Linie von Deutschland bis an die westliche Verbreitungsgrenze in den Niederlanden reicht. Diese Erkenntnis widerspricht bisherigen Annahmen, die von einer Differenzierung in Ost- und Westhamster in Mitteleuropa ausgingen (Mitchell-Jones et al. 1999). Wie schon in den beiden vorangegangen Publikationen angedeutet, erwies sich die westliche Population als Teil der nördlichen Hamstergruppe. Deren geringe genetische Variabilität resultiert aus einem Gründereffekt, der wahrscheinlich seinen Ursprung in der leptokurtischen Ausbreitung (Ibrahim et al. 1996) von einem großen, die mitteldeutschen Bördelandschaften umfassenden Hamsterareal hat. Dafür spricht auch die klare sternförmige Anordnung mitochondrialer Haplotypen in genetischen Netzwerken. Entsprechend den Fossilfunden in Deutschland, Frankreich und Belgien (Chaline 1972; Libois and Rosoux 1982; Storch 1987), die der heutigen *Cricetus*-Form zugeordnet werden, fand diese Migrationswelle im Spätpleistozän vor ca. 15.000 – 10.000 Jahren statt. Man geht heute davon aus, dass der Feldhamster, das letzte glaziale Maximum (20.000 – 18.000 BP) in weiten Teilen Mitteleuropas nicht überlebt hat. Es ist möglich, dass die Linie aus einem östlichen Areal expandierte und das glaziale Maximum lediglich eine zeitliche Barriere für die Besiedelung Deutschlands darstellte. Einfacher gestaltet sich die Interpretation der Daten für die pannonische Feldhamsterlinie. Das Auftreten von Fossilien in den Fundschichten von der Subalyuk-Zeit (~ 40.000 BP) bis in das

Holozän (Jánossy 1986) spricht für eine kontinuierliche Besiedlung des Karpatenbeckens seit ca. 40.000 - 50.000 Jahren. Die höhere genetische Diversität tschechischer und ungarischer Hamster sowie eine stärkere Strukturierung mitochondrialer Haplotypen deuten daraufhin, dass der Feldhamster sehr wahrscheinlich das letzte glaziale Maximum in den ungarischen Steppen überlebte.

Die Arbeit zeigt auch, dass die Verbreitungsmuster des Feldhamsters in Europa wahrscheinlich stark durch die Art und Weise der Ausbreitung beeinflusst wurden. Ein Hamster aus Polen ist genetisch deutlich unterscheidbar von deutschen Hamstern und eher mit russischen Haplotypen assoziiert. Das spricht für eine Besiedlung des nördlichen europäischen Areals in Wellen aus verschiedenen Quellpopulationen. Grund für diese höhere Dynamik der Populationsstruktur sind extremere Klimaeinflüsse auf den Feldhamsters im nördlichen Areal. Ein Feldhamster vom Balkan zeigt dagegen intermediäre Haplotypen zwischen pannonischen und russischen Tieren, was mehr auf eine stabilere Hamsterbesiedlung im südlichen Teil des europäischen Verbreitungsgebietes deutet. Eine deutliche Diversiät russischer Feldhamster steht mit der Existenz bedeutender glazialer Refugien im Hauptverbreitungsgebiet der Art im Einklang (Markova et al. 1995). Eine Differenzierung der mitteleuropäischen Feldhamster fand bereits vor der beginnenden Besiedlung vor ca. 85.000 - 147.000 Jahren im Kerngebiet der russisch-ukrainischen Steppen statt. Eine Analyse aller gefundenen Haplotypen, (Niederlande bis Westsibirien), gibt Hinweise für einen die Gesamtpopulation betreffenden Flaschenhals. Allerdings können homogenisierende Effekte durch genetische Drift nicht völlig ausgeschlossen werden. Eine Ausbreitung der Population könnte vor 118.000 – 204.000 Jahren im späten mittleren Pleistozän bzw. im Übergang vom Mittel- zum Jungpleistozän erfolgt sein. In dieser Zeit existierte eine Reihe von *Cricetus*-Formen in Europa und *Cricetus cricetus* drang bis nach Großbritannien, Italien und Israel vor (Schaub 1930; Kurtén 1968; Hír 1997; Jánossy 1986; Kowalski 2001). *Cricetus cricetus* erschien während des Eem, vor 115.000 – 135.000 Jahren, zum ersten Mal in Polen und Deutschland (Nadachowski 1989; Rathgeber und Ziegler 2003). Jedoch ist die Taxonomie der fossilen *Cricetus* umstritten und fossile Funde lassen sich häufig nicht eindeutig einer bestimmten Art zuordnen (Hir 1997). Problematisch ist dabei auch die morphologische Variabilität von *Cricetus cricetus* (Grulich 1987b, 1991). Eine große pleistozäne *Cricetus*-Form trat vor allem während der Interstadiale auf. Sie gilt inzwischen als nicht konspezifisch zu *C. cricetus* und wird als eigene Art *C. major* angesehen (Fahlbusch 1976).

Mit der Phylogeografie des Feldhamsters liegt eine erste Studie zu einem weit verbreiteten eurasischen Steppensäugetier vor. Sie unterstreicht die Bedeutung östlicher Glazialrefugien für die Besiedlung und Populationsstruktur Mitteleuropas und stellt eine wichtige Ergänzung zu bisherigen Phylogeografien dar, die die Bedeutung von Rückzugsgebieten im mediterranen Raum und dem Balkan belegen. Interessanterweise begründet sich die für viele europäische Tiere typische Nord-Süd-Verteilung mitochondrialer Matrilinien nicht in einer Immigration aus nördlichen und südlichen Refugien. Sie wird bestimmt durch die horizontale Anordnung geografischer Barrieren, wie z.B. die Gebirgskette der Karpaten, die Sudeten und die deutschen Mittelgebirge. Genetische Daten und Fossilfunde unterstützen die These, dass der Feldhamster wahrscheinlich ein Element der kühleren Klimaperioden ist. Extreme Kälte oder Wärmeperioden führten dagegen zur Aufgabe von Arealen außerhalb von Kernverbreitungsgebieten (Markova et al. 1995; Spitzenberger und Bauer 2001). Es ist durchaus möglich, dass der derzeit beobachtete rapide Zusammenbruch von Randpopulationen in Westeuropa auch eine Konsequenz klimatischer Veränderungen ist und nicht allein auf landwirtschaftlicher Intensivierung beruht. Ein Rückzug des Feldhamsters aus vorgeschobenen Arealen ist auch für andere Wärmeperioden des Holozäns belegt (Spitzenberger und Bauer 2001). Die Verbreitung des Feldhamsters in weiten Teilen Europas ist klar durch klimatisch-ökologische Faktoren begrenzt. Nur eine ausgedehnte Landwirtschaft ermöglichte die Besiedlung suboptimaler Gebiete. Andererseits wurden Bereiche, wie der nördliche Teil Polens, trotz landwirtschaftlicher Nutzung nicht durch den Hamster besiedelt. Auch die pleistozäne Verbreitung des Feldhamsters in Polen ging wahrscheinlich kaum über das heutige Areal hinaus (Nadachowski 1998).

Die unerwartete Populationsstruktur des Feldhamsters in Europa hat wesentliche Implikationen für den Feldhamsterschutz. Entsprechend den Konzepten und Richtlinien der IUCN für die Bewahrung evolutionär bedeutsamer Populationen, sollten sich zukünftige (und europaweite) Schutzkonzepte vor allem auf die Sicherung mitteldeutscher und pannonischer Hamster konzentrieren.

Die bestehenden Naturschutzkonzeptionen zu Grunde liegende Unterteilung in eine “westliche Unterart“ *C. cricetus canscens* und eine “östliche Unterart“ *C. cricetus cricetus* widerspricht den genetischen Befunden.

Die genetische Struktur rezenter Feldhamsterpopulationen nordwestlich des Karpatenbogens steht im Einklang mit einem Wiederbesiedelungsszenario nach dem letz-

ten glazialen Maximum. Allerdings kann ein Überleben der Art in weiten Teilen Mitteleuropas nicht gänzlich ausgeschlossen werden (Werth 1936; Pradel 1985). Aus diesem Grund wurde ein Projekt in Zusammenarbeit mit dem Max-Planck-Institut für Evolutionäre Anthropologie in Leipzig (Dr. M. Hofreiter) und dem Senckenberg-Museum in Weimar (Dr. L. Maul) begonnen, um genetische Analysen an Fossilien pleistozäner Feldhamsters durchzuführen. Dabei sollen vor, und unmittelbar nachdem glazialen Maximum auftretende genetische Haplotypen mit rezenten Daten verglichen werden.

3.4.1. 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 eleven 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 north-west 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 phylogeographic 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 - 147 ky ago. 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 phylogeographic units.

Das Konzept und das experimentelle Design wurden von mir entwickelt. Sämtliche genetischen Analysen erfolgten im eigenen Labor unter technischer Assistenz von G. Becke, B. Gebhardt und S. Hofmann. Dr. J. Michaux unterstützte einen Teil der statistischen Auswertungen. Das Manuskript wurde durch mich erstellt.

Although there are strong evidence that Pannonian hamsters have persisted inside the Carpathian basin over the last 50 ky, 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 Quarternary.

INTRODUCTION

The oscillation of warm and cold phases during the Quarternary has promoted extensive shifts of distribution areas, and hence population diversity, in many temperate animals (Cooper *et al*. 1995, Taberlet 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 and Searle 2002) with additional retreat areas in central Europe (Bilton *et al*. 1998, Brunhoff *et al*. 2003). Range expansion caused further genetic differentiation (Mila *et al*. 2000, Conroy and Cook 2000a) due to 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 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 characterised by several warm and cold (Dryas) spells. Spreading woods and increased humidity during the Atlantikum (7,500 – 4,500 BP) and early Sub-Atlantikum (2,800-2,000 BP) must have caused habitat losses for taxa adapted to arid continental climates.

Phylogeographic studies on small rodents have focussed mainly on species with relatively wide and northerly reaching distributions (Jaarola and Tegelström 1995, Jaarola and Searle 2002, Brunhoff *et al.* 2003). Despite similarities, such as the importance of southern European refugia, there is a large species-specific variance among phylogeographic 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 hiber-

nating animal lies in the eastern European and western Asian plains where it occupies steppe, meadows and steppe-forests (Nechay 2000, Berdyugin and Bolshakov 1998). The northern species boundary roughly coincides with 55° northern latitude, although in Russia it extends up to 59° northern latitude (Niethammer and Krapp 1982). Common hamsters in western and central Europe are largely restricted to agricultural sites with deep loess soils and suitable micro-climates (Grulich 1975, Nechay *et al*. 1977). Paleontological data suggest that *Cricetus cricetus* underwent repeated range shifts during the Quarternary (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 forty years (Backbier and Gubbels 1998, Murariu 1998, Nechay 2000). The most dramatic population collapses have occurred along the western frontier of the distribution (Libois and Rosoux 1982, Baumgart 1996). A previous study has shown that genetic depauperization of western hamsters, which are considered to represent the subspecies *C. c. 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 phylogeographic structure of the common hamster match that of other European muroids? Is it in agreement with a late/post glacial 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.

MATERIAL AND METHODS

Population sampling

Sampling mainly concentrated on populations in central and western Europe covering the range of the two subspecies *C. c. canescens* and *C. c. cricetus*. Additionally, we included hamsters from European Russia, as well as single specimens from each

of Romania, Poland and western Siberia. A total of 435 specimens were collected from more than 60 localities in 8 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).

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.

Sampling Region	Location (Country)	Sample ID	Analyzed individuals		
			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 Bassin / Pannonia	Southern Moravia (Czech Republic)	P1	65	24	65
	7 locations across Hungary	P2	40	25	40
Other samples 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

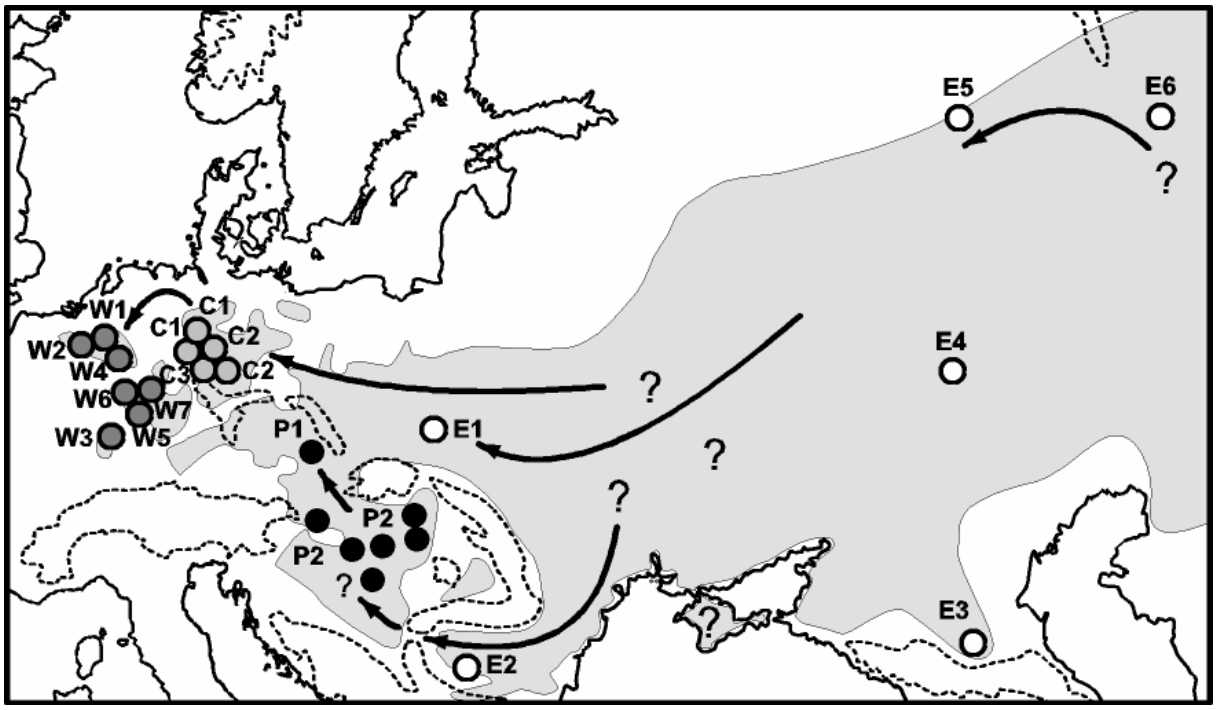

Fig. 1 Geographical distribution of *Cricetus cricetus* samples (circles) and proposed expansion routes (arrows). Grey areas refer to the recent distribution range according to Panteleyev (1998) and Mitchell-Jones *et al*. (1999). Question marks indicate potential glacial refugia deduced from fossil 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.

Mitochondrial DNA analysis

We investigated three partial mitochondrial (mt) regions; control region (*ctr*), 16SrRNA (*16S*) and cytochrome *b* (*cytb*). Before the experiment, we compared 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 and Igushima 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 as 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 (DIInt1: 5'-ATTCCCCTAGCATATAAGCAT-3', Ann.: 50°C; DIInt2: 5'-GTGGGCGGGTTGCTGGTTTCT-3', Ann.: 60°C).

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

Cytochrome *b* (984 bp total fragment length) was amplified with primers L14841 (Ko-

cher *et al*. 1989) and HCRIC3 (5'-GATGAAAGGGTATTCTACTGGTTG-3') at 50°C annealing temperature. Sequencing was carried out using flanking and internal primers (CbLint1: 5'-ACGTACTACCATGAGGTCAAAT-3', Ann. 51°C, CbLint2: 5'-TCCCGCACACATTAAACC-3', Ann. 50°C; CbHint1: 5'-GTGGATTTGCAGGAG-TATAAT-3', Ann. 50°C; CbHint2: 5'-AATGATTTGGCCTCATGGGAG-3', Ann. 53°C; CbHint3: 5'-CGGCAGATGTGGGTTACTGAT-3', Ann. 58°C).

Sequences were aligned in PROSEQ (v. 2.9, D.A. Filatov, University of Birmingham, UK) and the number of haplotypes (N_H) scored. Nucleotide diversity π (based on haplotypes in %) 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 with 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 and Nei 1993) and maximum-parsimony (MP: heuristic search, close-neighbour-interchange method with the random addition of 1000 trees; Nei and 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 *Fs* (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 and 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, University Park, Pennsylvania, USA). 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 PAUP4b5 (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 and Cook (2004) for *Microtus oeconomus.* Their rates were applied to combined *cytb* and *ctr* sequences and are based on divergence calculations obtained from two different Arvicolid genera *Lemmus* (Fedorov and Stenseth 2001) and *Microtus* (Conroy and Cook 2000b).

Microsatellite analysis

Hamsters were genotyped at eleven microsatellite loci (Neumann and Jansman 2004). Mean number of alleles (*A*) and observed heterozygosity (H_O) were calculated in GENEPOP (Raymond and Rousset 1995). Rogers' genetic distance *Dr* (Rogers 1972) between populations and population groups was computed in POPULATIONS. 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 *cytb* haplotypes (AJ633756-82) were identified in 45 individuals. 925 bp 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.

Table 2 Mitochondrial (N_H = haplotype number, π = nucleotide diversity) and microsatellite diversity measures of European common hamster phylogroups. Allel 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/Fs*) are based on pairwise mismatches of mt haplotypes.

Phylogroups (Sample ID)	**Mitochondrial Loci**			**Microsatellite Loci**		
	N_H (n) *ctr* *16S cytb* combined	***π*** ±*SE*	***Tau*** (95%CI) / ***Fs***	***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	-			
	1 (10)	0	-			
	5 (10)	0.1±0.1	3.00 (0.45-4.31), p= 0.560/ -3.83, p=0.001			
Central	3 (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				
	8 (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 (45)	1.2±0.2	13.83 (8.14-18.02), p=0.779/-19.33, p<0.001			
	27 (45)	1.1±0.2	26.43 (18.43-31.94), p=0.26/-10.58, p<0.001			

The numbers of informative sites were either lower than (*ctr*, *16S*) or equal (*cytb*) 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 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 *cytb* and *ctr* between North and Pannonia were rather similar (Pannonia: N_{Hctr} = 13, N_{Hcytb} = 13; π_{ctr} = 1.1 ± 0.3%, π_{cytb} = 0.6 ± 0.2% versus North: N_{Hctr} = 16, N_{Hcytb} = 9; π_{ctr} = 0.9 ± 0.3%, π_{cytb} = 0.4 ± 0.1%). Pannonian hamsters proved invariant for *16S* unlike all other populations. D_a values between the two phylogeographic groups were as $D_{a\ ctr}$ = 1.0 ± 0.4%, $D_{a\ 16S}$ = 1.3 ± 0.5%, $D_{a\ cytb}$ = 0.9 ± 0.3%, $D_{a\ \text{comb.}}$ = 1.1 ± 0.3%.

Both main lineages show signs of further sub-structuring. 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%, *cytb* = 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 building methods confirm a significant separation of Hungarian and Czech hamsters within the Pannonian lineage and show almost identical topologies.

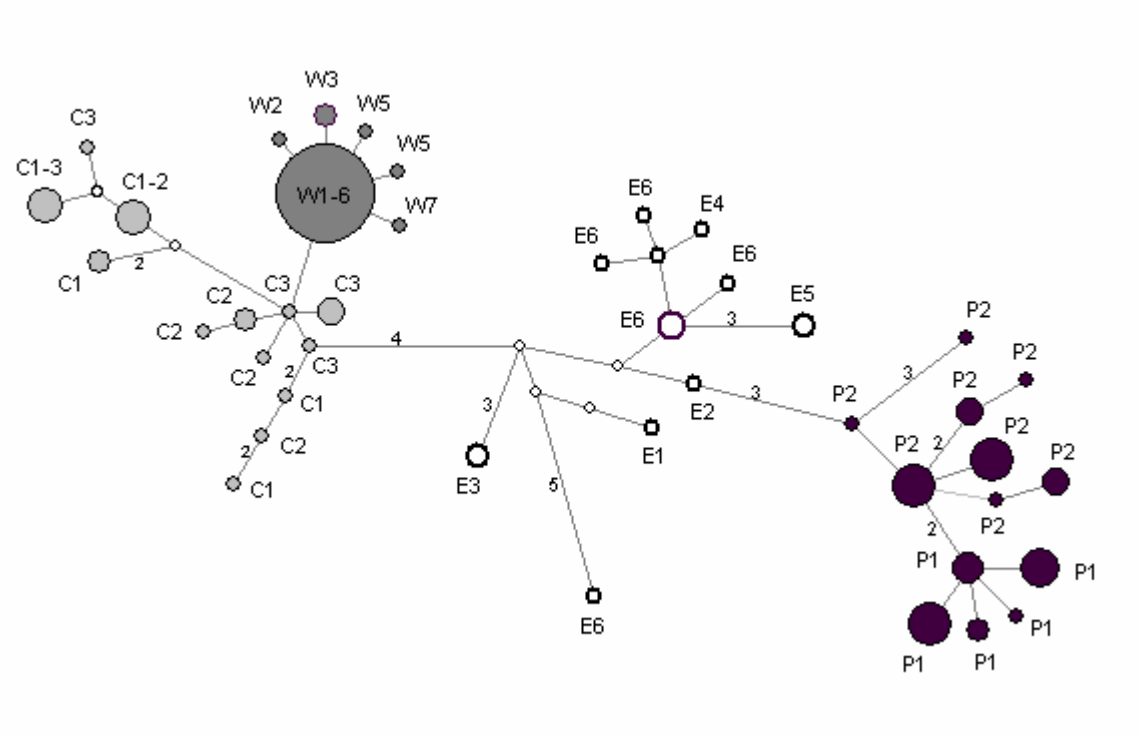

Fig. 2 Median-joining network based on combined *ctr* + *16S* haplotypes (n = 43) obtained from European common hamsters (n = 130). Small empty circles refer to missing intermediates with relevance for construction of links. Links were modified in cases where more than one connection was possible. Numbers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

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 phylogeographic 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 is close to the northern species boundary. A 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 *Fs* rejects constant size for single genes ($p \leq 0.001$) and combined genes (p = 0.017 - 0.001).

The Relative Rate Test indicated no significant rate heterogeneity between phylogeographic groups or between genes (*ctr*: Z = 1.344, *16S*: Z = 1.344, *cytb*: Z = 0.380, all not significant) using the K2P distance. To carry out the test we compared 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 (π_{ctr} = 1.5 ± 0.3%, π_{16S} = 0.9 ± 0.3%, π_{cytb} = 1.2 ± 0.2) allows their combination for time estimates. Based on that, we obtained the following molecular datings: 85 - 147 ky ago (95%CI: 39 – 225 ky) for the split between North and Pannonia, 37 – 64 ky (95%CI: 17 – 102 ky) for the expansion of North and 25 – 44 ky (95%CI: 11 – 112 ky) for the expansion of Pannonia.

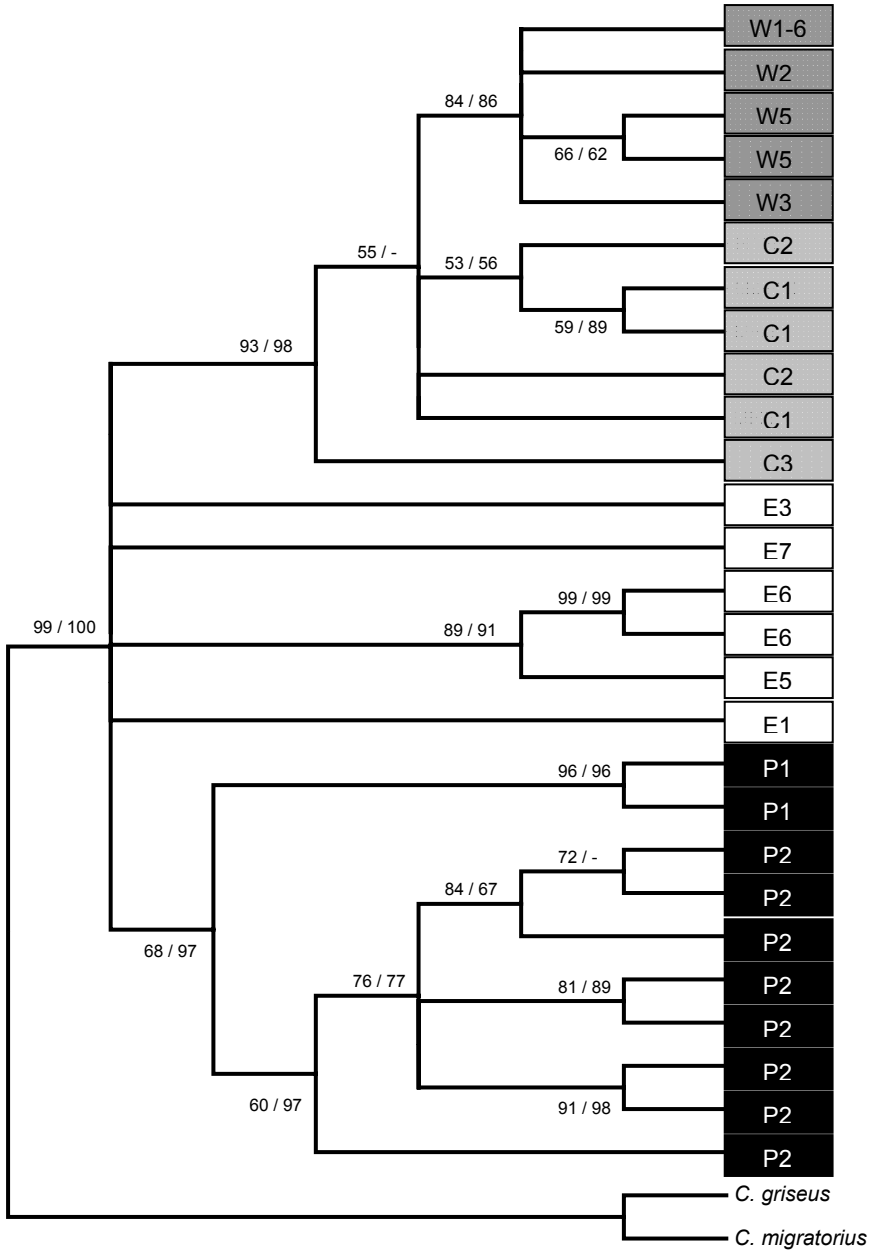

Fig. 3 Consensus ME tree based on 27 combined mt haplotypes (*ctr* + *16S* + *cytb*) of 16 common hamster populations. Numbers on branches correspond to boostrap support (1000 replicates; ME/MP). Haplotypes of two related hamster species *Cricetulus griseus* and *Cricetulus migratorius* served as outgroups. Labels are as in Table 1.

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, *Ccr*µ3 and *Ccr*µ6, 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 tetra-nucleotide

locus *Ccr*µ6 occurs 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 ± 0.02 and 0.69 ± 0.04, respectively. West is much less heterozygous H_o = 0.38 ± 0.06.Pannonia and North exhibit high observed heterozygosity values with H_o = 0.76 ± 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 ± 0.06; Central: 0.69 ± 0.04). North shows a slightly smaller allele number (A = 11.55 ± 1.53) and allele size range (R = 10.5 ± 0.13) than Pannonia (A = 13.27 ± 1.34; R = 15 ± 1.81). Diversity of the single representative eastern population from the Ural (E6, n = 19) was H_o = 0.61 ± 0.04, A = 5.45 ± 0.47, which is comparable to the means of the entire eastern sample (H_o = 0.60 ± 0.04, A = 7.4 ± 0.45, n = 24).

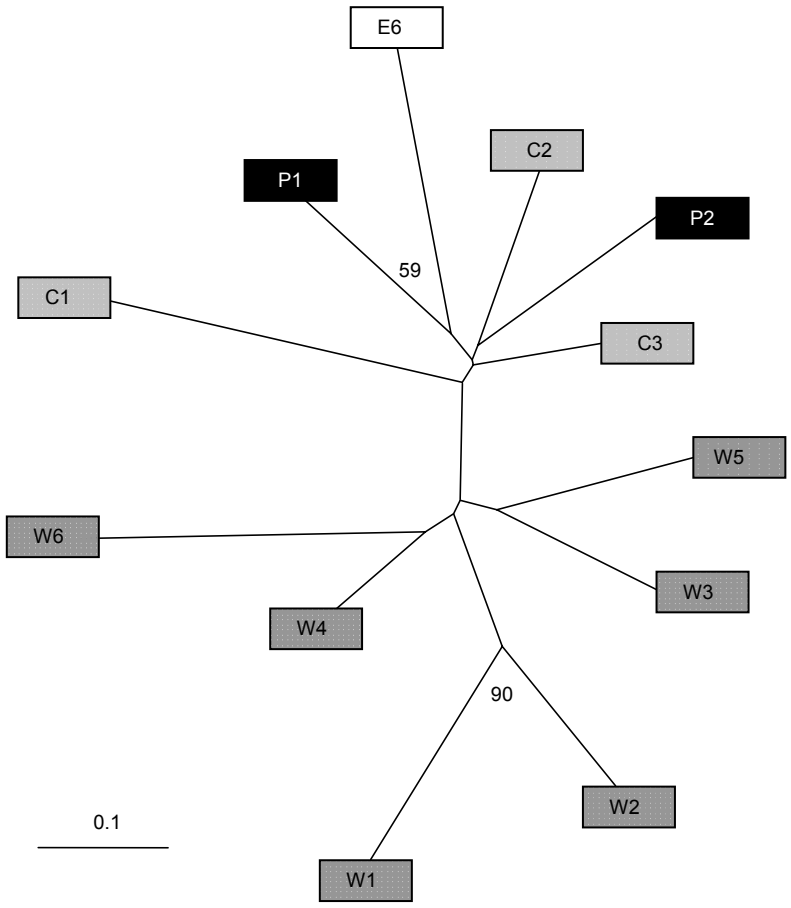

Fig. 4 Neighbour-joining tree based on 11 microsatellite loci comprising 12 European common hamster populations (Rogers genetic distance). Numbers on branches represent bootstrap support (1000 replicates; based on number of loci). For labels see Table 1.

DISCUSSION

Glacial history and the timing of population differentiation

The phylogeographic 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 phylogeographic structure found in other European muroids (Jaarola and 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 paleontological data is slightly hampered by the differing opinions about taxonomic relationships within the genus *Cricetus* (von Königswald 1981, Pradel 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 *Cricetus 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, as a result of the unsuitable paleoclimatic and paleogeographic conditions (Lozek 1973, Kahlke 1981). In contrast, Janossy (1986) and Hir (1997) showed an almost uninterrupted record of *Cricetus cricetus* for Hungary from the lower Weichselian (Subalyuk, ~40.000 BP) onwards.

Our molecular clock estimates suggest that the separation between North and Pannonia occurred around 85 - 147 ky ago (95%CI: 39 – 225 ky). This time window encompasses a period of major temperature shifts, including the entire Eem interglacial (~135,000 – 115,000 BP). Fossils predict a first appearance of *Cricetus cricetus* in central Europe at the beginning of the Eemian (e.g. Rathgeber and 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 (~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 recoloni-

zation of central Europe. Expansion times of 37 – 64 ky (95%CI: 17 – 102 ky) for North and 25 – 44 ky (95%CI: 11 – 112 ky) for Pannonia fall inside the Weichselian (115,000 – 10,000 BP). This finding is concordant with the repeated appearance of *Cricetus 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 *Cricetus 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 - 50 ky. 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, since 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's 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 *cytb* + *ctr* only; 34 – 60 ky, 95%CI: 16 – 154 ky).

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 and Krapp 1982, Nechay 2000). Markova *et al*. (1995) could show from fossil

records that, at the end of the last glaciation, the Russian hamster range contracted to the west with the Ural 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 lineage North. 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 ky (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 phylogeographic pattern that differs from those previously found for other small mammals (Bilton *et al*. 1998, Jaarola and 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 – 204 ky ago, 95%CI: 68 – 266 ky) 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 1995, Jarne and Lagoda 1996) explaining the lack of resolution when using allele frequencies to discriminate hamster populations. In contrast, a specific allele spacing pattern at locus *Ccr*µ6 and the unexpected sequence variant at locus *Ccr*µ3 are very likely identical by descent. The 192 bp allele at locus *Ccr*µ3, 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 readily be explained by the much larger effective population size compared to maternally inherited genes (Zhang and 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 geographic 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 due to 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 geographic 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 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 due to suitable micro-climatic 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 and 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, which 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 phylogeographic 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 mt DNA phylogenetic lineages in central Europe does not correlate with the proposed existence of a western subspecies *C. c. canescens* and an eastern form *C. c. 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*. (2004) reporting overlapping cytochrome *b* haplotypes between Dutch and Czech hamsters, but their study experienced some irregularities in the methodology (Smulders pers. comm.).

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 forty years. Dramatic declines of hamster populations in the Netherlands (Backbier and Gubbels 1998), Belgium (Mer-

celis 2002) and the extinction of northerly populations in Germany (Krüger and 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 e.g. 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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4. Genetische Variabilität und Populationsstruktur des Goldhamsters *Mesocricetus auratus* (Waterhouse)

4.1. Der Goldhamster *Mesocricetus auratus*

Die Gattung *Mesocricetus* umfasst vier Arten *M. auratus*, *M. raddei*, *M. brandti* und *M. newtoni* (Wilson und Reeder 1993; Duff und Lawson 2004). Wenige Studien beschäftigen sich mit der Evolution der einzelnen Arten und deren verwandtschaftlichen Beziehungen (Hamar und Schutowa 1966; Hosey 1982; Niethammer 1982). Neue Erkenntnisse dazu wurden bereits im Kapitel über die Systematik der Cricetinae diskutiert.

Die mit Abstand bekannteste Hamsterart ist der Goldhamster *M. auratus* (Abb. 1). Seine Popularität verdankt er seiner guten Eignung als Labortier aber auch seiner Attraktivität als Heimtier. Kurze Generationszeiten und anspruchslose Haltung machten ihn zu einem wichtigen Versuchstier für die biologische und medizinische Forschung (Clark 1987).

Abb. 1 Goldhamster (Foto: K. Neumann)

Besondere Bedeutung erlangte der Goldhamster für die Chronobiologie (Lowrey et al. 2000), für die er als Modelltier gilt. Bemerkenswert ist auch die Historie der Laborpopulation des Goldhamsters. Die gesamte Laborpopulation geht auf drei Nach-

kommen eines Weibchens zurück, das 1930 in der der Nähe von Aleppo/Syrien gefangen wurde (Aharoni 1932, Gattermann 2000). Die Gefangenschaftspopulation beträgt inzwischen mehrere Millionen Tiere. Kein anderes Säugetier erzielte einen solchen Reproduktionserfolg ausgehend von einem so kleinen Genpool. Deshalb wird der Goldhamster mitunter als Musterbeispiel für die erfolgreiche Etablierung einer stabilen Population trotz hoher genetischer Inzucht angeführt (u.a. Frankham et al. 2000). Der geringe genetische Polymorphiegrad der Laborhamster (McGuire et al. 1985; Watkins et al. 1990) schränkte allerdings deren Nutzung für molekulargenetische Untersuchungen wie Genom-Sequenzierungen oder Kartierungs-Experimente interessanter Loci entscheidend ein (Okuizumi et al. 1997).

Obwohl eine große Zahl von Studien und biologischen Daten zur Laborpopulation existiert, gibt es kaum Erkenntnisse zur Ökologie oder Populationsgenetik des Goldhamsters im Freiland. Dies ist insbesondere problematisch, da eine Bewertung bekannter biologischer Phänomene der Laborhamster wie z.B. eine stabile Aktivitätsrhythmik (Tagesruhe/Nachtaktivität), relative Inzuchtresistenz oder geringe Transplantatabstoßung (kaum MHC-Variabilität) nur im Vergleich mit der Wildpopulation erfolgen kann. Die wenigen bisher dazu vorgenommenen Studien beschränken sich auf den Vergleich von herkömmlichen Laborzuchtlinien mit einer zweiten, von Murphy 1971 etablierten Linie. Die Murphy-Linie ging aus 11 Tieren hervor, die in Syrien gefangen und nach Amerika verbracht wurden. Tiere dieser inzwischen erloschenen Linie fanden keinen Eingang in den Haupt-Genpool der Laborpopulation. Daten zur Ökologie und Verbreitung des Goldhamsters sind anekdotenhaft und beschränken sich auf wenige Expeditionsberichte (Aharoni 1932; Murphy 1971 und 1985; Lyman und O'Brian 1977). Deshalb wurden zwei Expeditionen nach Syrien (1999, 2000) und drei weitere in die Türkei (2002, 2003, 2005) durchgeführt, um neue Erkenntnisse zur Verbreitung, Verhalten und Ökologie des Goldhamsters zu gewinnen. Die Etablierung eines neuen Wildstammes als auch die Sammlung von Gewebeproben ergab dabei die Möglichkeit vergleichende morphologische, ethologische und genetische Untersuchungen zu konzipieren.

Publikation V: „Notes on the current distribution and the ecology of wild golden hamsters (*Mesocricetus auratus*)" berichtet von der Wiederentdeckung des Goldhamsters in Nordsyrien. Es werden klimatische Daten, Bodenparameter und Habitatbeschreibungen für die vom Goldhamster besiedelten Gebiete angegeben. Gleichzeitig wurde die Baustruktur der Art ausführlich untersucht. Anhand der Aus-

wertung eigener und publizierter Funddaten wurde die erste genauere Verbreitungskarte für die Art publiziert. Neben der Rolle von Prädatoren werden Informationen zu anthropogen bedingten Gefährdungsursachen für den Goldhamster diskutiert. Die im Jahre 2000 gefangenen Tiere bilden den Grundstock eines neuen Zuchtstammes am Institut für Zoologie der Martin-Luther-Universität Halle-Wittenberg für vergleichende Untersuchungen.

4.1.1. Notes on the current distribution and the ecology of wild golden hamsters (*Mesocricetus auratus*)

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Abstract

Two expeditions were carried out during September 1997 and March 1999 to confirm the current existence of *Mesocricetus auratus* in northern Syria. Six females and seven males were caught at different sites near Aleppo. One female was pregnant and gave birth to six pups. Altogether, 30 burrows were mapped and the structures of 23 golden hamster burrows investigated. None of the inhabited burrows contained more than one adult. Burrow depths ranged from 36 to 106 cm (mean 65 cm). Their structure was simple, consisting of a single vertical entrance (gravity pipe) that proceeded to a nesting chamber and at least one additional food chamber. The mean length of the entire gallery system measured 200 cm and could extend up to 900 cm. Most burrows were found on agricultural fields preferentially on leguminous cultures. The distribution of golden hamsters is discussed in association with historical data, soil types, geography, climate and human activities.

All 19 golden hamsters were transferred to Germany and, together with three wild individuals supplied by the University of Aleppo, form a new breeding stock.

INTRODUCTION

The Syrian or golden hamster *Mesocricetus auratus* (Waterhouse, 1839) is one of the best-established experimental animals and probably among the most popular pets in the western world. A wealth of data on the behaviour, chronobiology, immunology and physiology of the species have been obtained from laboratory-bred individuals.The entire laboratory population of golden hamsters originated basically from a

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Die hier veröffentlichten Daten wurden während der von Prof. R. Gattermann organisierten Expeditionen gesammelt. Biologische und Verbreitungsdaten zum Goldhamster wurden von den Autoren gemeinsam erhoben. Ich war sowohl an der Auswertung der Daten als auch an der Erstellung des Manuskriptes beteiligt.

single brother-sister pairing in 1930 (Aharoni, 1932) with the exception of 12 wild animals brought to the U.S.A. in 1971 (Murphy, 1985). Since then only a few golden hamsters have been caught in Syria and Turkey (Table 1). In contrast to the popularity of the hamster, virtually no data exist concerning its ecology, population genetics or even its recent occurrence in the wild**.** This is partially because of its narrowly restricted distribution area. Two expeditions were organised to furnish proof of the existence of golden hamsters in northern Syria and if possible to acquire wild animals for behavioural and genetic studies.

MATERIALS AND METHODS

Joint expeditions by the universities of Halle (Germany) and Aleppo (Syria) were led to northern Syria in late summer 1997 and spring 1999. The aim of the first expedition (30 August – 17 September 1997) was to explore the presumed distribution area around Aleppo and to identify suitable hamster habitats. The hamster search included the location of used burrows and an attempt to catch animals with live traps baited with apple and melon pieces. Interviews with village elders and economists were carried out. A photograph of the golden hamster was shown to them and the interviewees had to describe the typical features of a golden hamster burrow as well as morphological characteristics of the animal itself, including differences from other related species e.g. the grey hamster *Cricetulus migratorius*.

The second expedition from 4 to 27 March 1999 was led to the most promising hamster sites. Twenty-three burrows were excavated, measured and if possible the animals caught. Means and standard deviations of all measurements are presented. Additionally, a typical soil profile was drawn up in the region near Azaz. The characterization of the soil horizons followed Scheffer & Schachtschabel (1998) and Spaagaren (1994). Two data loggers (OTLM Gemini Data Loggers (UK) Ltd.) were used to monitor the air temperature 70 cm above the ground and the soil temperature at a depth of 70 cm. Registration was carried out every 12 min. The locations of all hamster burrows were assessed via a GPS-location system (GPSMS1 from µ-blox Switzerland).

Table 1 List of historic and recent distribution records of golden hamster (*Mesocricetus auratus*). # refers to the map-location (see Fig. 1), (?) unconfirmed reference.

#	Location	Date	Reference	Remarks
1	Aleppo (?)	1797	Russel & Russel, 1797	Earliest description of the Syrian golden hamster
	Aleppo (?)	1839	Waterhouse, 1839	Type specimen, probably caught by the Russel brothers
	Aleppo (?)	1880	Reynolds, 1954	J.H. Skene, Consul General at Aleppo brought living hamsters to Britain
	Aleppo	June 1902	Nehring, 1902	1 preserved ♀was sent to Berlin by Zumoffen (Beirut)
	Aleppo	12 April 1930	Aharoni, 1932	1♀ and 11 juveniles were excavated by I. Aharoni, 3 ♂♂ and 1♀ are the ancestors of all captive golden hamsters
	Aleppo (?)	1962/1972	Kumerloeve, 1975	3 hamsters were caught and sent to Turkey (1) and to the USA (2)
	Aleppo	Autumn 1982	Henwood, 1999	1♂ and 1♀ were caught, ♀ was brought alive to London, cross-pairing with laboratory hamsters failed
	Aleppo	May/June 1971	Murphy, 1971	13 hamsters were trapped, 4 ♂♂ and 8 ♀♀ were transferred to the USA and separate breeding stock established (Coe & Ross, 1997)
	Aleppo	1978	Murphy, 1985	2 ♀♀ were brought to the USA by B. Duncan
2	Biliramun	April 1930	Aharoni, 1932	Further 3 ♀♀ were collected by I. Aharoni, the skulls are in Berlin
3	Azaz	April 1930		
4	Antakya (?)	Spring 1949	Eisentraut, 1952	According to Eisentraut a gravid E was caught 20 km east of Antakya and 2 ♂♂ offspring were taken to Germany. This finding was not confirmed by others and has to be questioned.
5	Jarablus	1986	Tichy, 1998	3 ♂♂ were brought to Tübingen (Germany), no reproduction success
6	Kesiktas	July 1991, 1996, 1997	Dogramaci et al., 1994 Kefelioglu, 1999	4 hamsters were collected for taxonomical studies
7	Kilis	Spring 1999	Yigit ,1999	1 ♂ and 1 ♀ were sampled for taxonomic studies, further records in Yigit et al., 1997
8	Albel	March 1999		7 ♂♂ and 6 ♀♀ caught at Albel (3,2), Shaykh-Riek (1,1) and Arnaz (3,3); 30 burrows were mapped and 18 completely measured. The hamsters were transported to Halle (Germany) and form the source of a new breeding stock.
9	Shaykh-Riek			
10	Arnaz			

RESULTS

Animals

Altogether 13 hamsters, seven males and six females, were caught at two locations near Albel/Shaykh-Rieh and Arnaz about 50 km north-east and 20 km south-west of the city Aleppo, respectively (Tab. 2). Average adult body weight measured 99.5 ± 5.9 g (±SD) for males (n = 6) and 76.0 ± 13.7 g for females (n = 3). One female was pregnant (excluded from body measurements) and soon gave birth to 6 offspring. Three 2- to 3-week-old juveniles (one male and two females) were found inside burrow 30, which had been used for several years. There were no obvious morphological differences in comparison to laboratory animals except that the coloration of wild animals appears slightly more intense.

Eleven animals were excavated from their burrows. Two other individuals were trapped by local farmers by flooding the burrows. Only one female showed several scabbed bite marks on her back. All other hamsters were in good physical condition without injuries or obvious bite marks and were free of ectoparasites.

Burrow structures

Thirty hamster burrows were found and mapped. Twenty three were excavated and measured. Complete data are only available for 18 burrows, since not all the tunnels in the remaining burrows were detected. Fully excavated burrows without hamsters were categorised as hamster “absent” if clear signs of activity (e.g. fresh green plant material) were detected or as “deserted” when lacking such signs (Tab. 2).Burrow depths varied between 36 and 106 cm and averaged 64.8 ± 17.6 cm. The mean total tunnel length was 199.5 ± 92.6 cm and could range for > 9 m (burrow 30). The burrow entrances measured 4 - 5 cm in diameter and led into a vertical tunnel of 18 - 45 cm length - the “gravity pipe”. Occupied burrows were always plugged with a lump of earth, which was missing in unused burrows. On average the sealing was placed about 22 cm below the surface. The smallest plug extended only 5 cm but some were up to 10 cm. After the gravity pipe, the tunnel levelled out and continued at a slight angle further downward to the nest chamber. The 10- to 20-cm-wide nest chamber was located 58.3 ± 12.7 cm below the surface. Its interior consisted of a spherical nest made of dry plant material. Two nests included textile remnants, bird feathers and shredded plastic sack pieces in the nesting material. At least two tun-

nels divided from the chamber. A 10 - 15 cm blind-ending tunnel was apparently used for urination. Faeces were found throughout the entire burrow. The remaining tunnels measured about 100 - 150 cm and ran deeper at varying angles and were partially used for food storage. Ten burrows contained a varying amount of green plant material such as chickpea and were therefore considered as inhabited. In three deserted burrows, only old or rotten grain (barley, weed) was found. The remaining burrows were empty.

Table 2 Measurements of golden hamster burrows and trapped inhabitants. Burrows with incomplete data (*) were not included in all calculations.

Burrow	Total tunnel length (cm)	Max. Depth (cm)	Depth of nesting chamber (cm)	Entrance Ø (cm)	Gravity pipe length (cm)	Depth of the clot (cm)	Inhabitant, body weight, location and remarks
# 1	90	62	60	4,5	34	20	ad. ♀, 103 g, Albel
# 3	270	57	50				deserted, Albel
# 4	220	55	54	4	19	19	ad. ♂, 88 g, Albel
# 5	270	48	48	4,5	18		deserted, Albel
# 6	170	75	65			None	absent, Albel
# 7							ad. ♂, 93 g, Albel; floated to the surface
# 8							ad. ♂, 67 g, Albel; floated to the surface in bad condition
# 10	100	36	36	4			deserted, Albel
# 12	215	106	55	4,5	25	15	ad. ♂, 97 g, Albel
# 13*		> 90		4	35	17	not found, Albel
# 14	126	65	65		36	36	ad. ♂, 99 g, Arnaz
# 15	235	53	45	4			deserted, Arnaz
# 17	150	70	none	5	38		deserted, Arnaz
# 18	177	70	70	4	45		deserted, Arnaz
# 19*	> 150	70	50	5		30	ad. ♂, 114 g, Arnaz; gravid
# 20	363	69	60	4,5	26		deserted, Arnaz
# 21*	> 180	80		5		25	not found, Arnaz
# 22*	> 285	93	93	4,5	24	24	not found, Arnaz
# 23	130	58	53	4	25		deserted, Arnaz
# 25	105	60	none	4	15	15	ad. ♂, 58 g, Shaykh-Rieh
# 26	130	100	none	4		20	ad. ♂, 92 g, Shaykh-Rieh
# 27*		63	63	4,5	33	14	not found, Shaykh-Rieh
# 28	220	47	47	4,5		23	ad. ♂, 128 g, Arnaz
# 29	420	70	70	5	25		deserted, Arnaz
# 30*	>900	85	65	5	25	17	Juveniles: ♂, 30 g, ♀, 23 g, ♀, 29 g, Arnaz
Mean	199.5	64.8	58.3	4.4	28.2	21.2	
SD	92.6	17.6	12.7	0.4	8.3	6.4	

Three deserted burrows were being used by green toads *Bufo viridis*. No differences between female and male burrows were detected. However, the largest and most complex burrow excavated contained three juveniles and apparently belonged to a female with her litter. The burrow density for the agricultural fields around Azaz could only be estimated. Fifteen burrows (six occupied, nine “empty”) were located in an area of 30 ha. The shortest distance between burrows measured 38 m. However, the closest distance between occupied hamster burrows was 118 m. Grassy embankments exhibited higher burrow densities but the degree of occupation could not be assessed.

Habitat and geographical distribution

Burrows were found mainly in fields with annual crops comparable with the preferences of common hamsters *Cricetus cricetus* in Europe. Most frequently these were weed, barley, chickpea, lentil and fruits and vegetables such as melon, tomato, cucumber and hibiscus. Fields had to be irrigated depending on the type of culture. Normally, 2 years of cereal crop are followed by a single year of leguminous cultures. Refuge areas like barren, bushes or hedges were often missing as a result of the increasing urban spread and extensive farming. Even ridges to mark the field boundaries of neighbouring villages were restricted. Only roadsides and narrow barren stripes around irrigation wells remained as alternative hamster sites. The main distribution area (Fig. 1) of the golden hamster lies in the fertile, agricultural and densely populated Aleppinian plateau in Syria, 280 - 380 m above sea level. The area covers only 10,000 – 15,000 km^2 and ranges north and south-west of the city of Aleppo. The North-Syrian limestone massif and the Turkish Taurus mountains form the natural western and northern barriers. The River Euphrates limits the range to the east and the stony steppe can be considered as an invincible barrier in the south-east. The south limit has not yet been defined but may reach as far as the beginning of the Syrian desert.In addition to our observations, sightings of the golden hamster have been reported from Jarablus in Syria, and Kilis and Kesiktas (near Gaziantep) in Turkey (Dogramaci, Kefelioglu & Gunduz, 1994; H. Kefelioglu, pers. comm.; N. Yigit, pers. comm.; Table 1, Fig. 1).

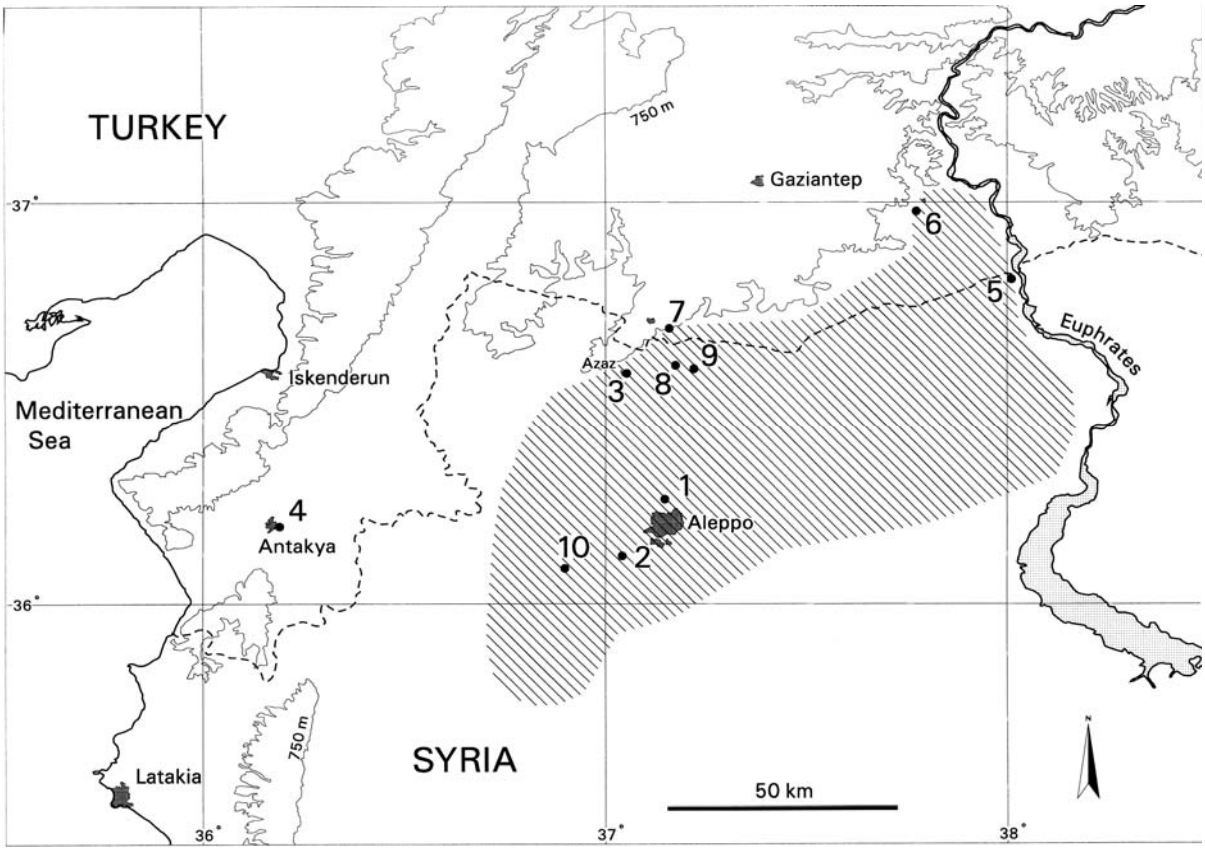

Fig. 1: Distribution map of the golden hamster (*Mesocricetus auratus*). Numbers indicate historic and recent records according to literature and personal communication (see Tab. 1)

Climate and soil conditions

The climate of the studied area was continental with large seasonal and diurnal fluctuations (Fig. 2) and low rainfall of 336 ±78 mm/year. The winter season was wet and cold with temperatures of *c*. 10 °C. There were occasional spells of frost or snow. The annual number of frosty days averaged 35.2, with absolute minimum temperatures of – 4 to – 9 °C (Anonymous, 1991 - 92). Based on our data, temperatures in August and September reached 35 - 38 °C at midday and 30 - 32 °C close to sunset. Along with shading light and the beginning of the hamsters' surface activities (according to laboratory observations), temperatures fall rapidly to 15 °C at midnight and *c*. 6 °C immediately before sunrise. March temperatures varied between 4.6 and 18.4 °C, 70 cm above ground. In contrast, below-surface measurements revealed an almost constant temperature of 12 °C. Only low fluctuations from 11.9 to 12.2°C were detectable at a depth of 70 cm, where the nesting chamber usually lies.

Soils based on sandy clay materials overlaying limestone are the dominant soil types found in hamster areas. All excavated burrows were on light-brownish chromic cambisols (terra fusca) or red rhodochromic cambisols (terra rossa). Both soil types have

a high clay component and the resulting high plasticity provides optimal conditions for fossorial animals. Table 3 shows a chromic cambisol profile with the typical structure found in the regions. The potentially available water capacity is high in comparison to most soil types found in Central Europe. However, the actual capacity is probably much lower because of the dry climate. The clay-rich soil exhibits low water conductivity, particularly in the less rooted subsoil.

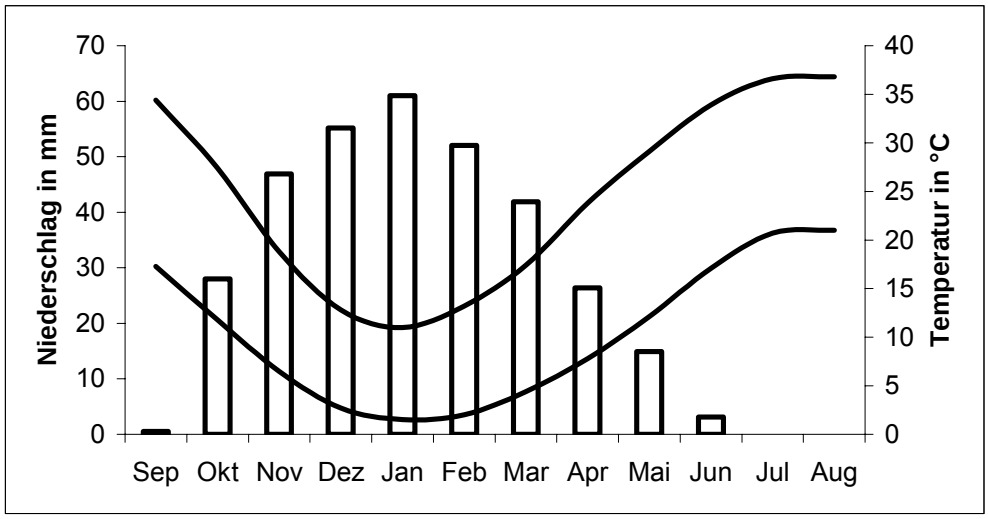

Fig. 2: Maximum and minimum temperatures (lines) and total precipitation (bars),for the natural distribution area of the golden hamster in northern Syria. Mean values are given for the period of 1978 to 1992. Data were provided by the meteorological station Tel Hadya, about 35 km south-west of Aleppo (Anonymous 1991/92).

DISCUSSION

During the two short stays in the main distribution area of the golden hamster, only a limited number of biological data could be obtained. Nevertheless, our findings disprove occasional opinions that the golden hamster has become extinct and the existence of *M. auratus* populations was confirmed.

Aharoni (1932) reported *M. auratus* from three different locations in Syria (Aleppo, Biliramun, Azaz) and Murphy (1971) collected hamsters near Aleppo. We found golden hamsters at two sites about 19 km south-west of Aleppo and 13 km east of Azaz, respectively. Another three males were recorded near Jarablus 90 km east of Azaz (H. Tichy, pers. comm.). Two known populations on the Turkish side exist at Kilis (15 km north of Azaz) and near Gaziantep (54 km north of Azaz). The Turkish and Syrian sites may form a connected distribution area, but data linking the currently known populations are missing e.g. from the military protected border zone between Syria and Turkey.

The natural habitat of the golden hamster is described as rocky steppe or brushy

slopes (Clark, 1987). In contrast, almost all our golden hamsters were excavated from burrows on agricultural land. The search for signs of hamster activities in steppe areas around the town of Afrin during summer 1997 ended without success. This corresponds with Aharoni (1932), who described the species from cultivated grain fields. Most burrows were found on plots with leguminous cultures. This may be an indication of existing preferences. Others found *M. auratus* on grassy embankments (Reynolds, 1954; Harrison, 1972). In fact we obtained a single individual from such sloping ground and identified several burrows on embankments near irrigation wells. To our knowledge there is no evidence for current sightings of golden hamsters in true steppe habitats in Syria. Literature reports about steppe populations probably result from confusions with the Turkish hamster *Mesocricetus brandti,* a rather similar species occurring in many other countries of the Near East. The golden hamster, like many steppe animals such as the common hamster *Cricetus cricetus* in middle Europe, has developed a preference for the abundant, food rich and optimal ground conditions provided by agricultural sites in northern Syria. The destruction of natural steppe habitats in Syria has certainly accelerated this process of adaptation. The species distribution is presumably patchy but the animal may be locally abundant according to our own observations and those of local farmers.

Table 3 Soil profile taken near Albel. Horizon description and colour follows Scheffer & Schachtschabel (1998) and Spaagaren (1994).

No	Horizon	Depth (cm)	Colour	pH ($CaCl_2$)	$CaCO_3$ (%)	Density (g/cm^3)	Sand (%)	Silt (%)	Clay (%)	Structure	Fine roots (per dm^2)
1	Agric	- 25	5YR 3/6	7.41	2 – 4	1.25 – 1.45	0 – 45	0 – 20	40 – 60	granular	6 – 10
2	Chromic cambic 1	- 60	5YR 4/6	7.39	2 – 4	1.45 – 1.65	0 – 45	0 – 20	40 – 60	subangular blocky	3 – 5
3	Chromic cambic 2	- 96	5YR 5/6	7.50	2 – 4	1.45 – 1.65	45 – 65	0 – 10	35 – 55	subangular blocky	1 – 2
4	Calcaric	> - 96	5YR 7/4	7.32	> 10	> 1.85	65 – 80	0 – 20	20 – 35	-	0

Previously published burrow structures by Herter & Lauterbach (1955), Dieterlen (1959) and Ropartz (1962) have been obtained under laboratory conditions, e.g. limited space, and do not entirely agree with our measurements. The sole data of a natural hamster burrow belong to that excavated by Aharoni in 1930, who described the location of a nest with pups at a depth of 2 - 2.5 m (Aharoni, 1932, Aharoni 1942). These data are not in concordance with our findings either (Table 2) and may represent an extreme value. The relatively simple structure of the golden hamster burrow

is rather different from those of common hamsters *C. cricetus*, which often exhibit > 10 branches (Grulich, 1981). The lack of variation between male and female burrows may be due to the early breeding season. For common hamsters it has been reported that sex-specific differences in burrow structures are only observed in female burrows depending on whether they contain litters (Grulich, 1981, Weidling & Stubbe, 1998). This could explain the exceptional structure of burrow 30 (Tab. 2).

Only a single adult golden hamster was found in every burrow, which may be evidence that they are solitary in the wild, supporting the general characterization of this species. Laboratory studies have shown that artificial grouping leads to symptoms of stress (Gattermann & Weinandy 1996 - 97).

According to local farmers, hamsters disappear in November and show first signs of activity at the beginning or middle of February. Whether these observations can be interpreted as an indication of the existence of a hibernation period remains unclear. In laboratory experiments, hibernation could be induced by keeping golden hamsters at temperatures below 8 °C (e.g. Smit-Vis & Smit, 1963; Ueda & Ibuka, 1995). Unfortunately, no long-term soil temperatures for the depth of the burrows were available for northern Syria, but air temperatures may drop well below 0°C during the winter. No data concerning the reproduction of golden hamsters in the wild exist. However, the presence of 2- to 3-week-old juveniles in one of the excavated burrows and the capture of a gravid female which gave birth on 24 March indicate that reproductive activity may start as early as February. This falls well within the time of the animals´ reappearance according to our questionnaire. During these interviews the rural population repeatedly mentioned gradations and the last appearances, which occurred in 1995 around Azaz.

Natural predators of hamsters such as foxes, mustelids or owls are scarce or hunted down. The same applies to larger reptiles or snakes. Other birds of prey may only occasionally take a golden hamster due to its nocturnal behaviour, but the presence of hamsters in their diet cannot be quantified since no data are available. Overall, the impact of natural predators on hamster populations can probably be ignored. Stray dogs are abundant but probably do not endanger golden hamsters. In contrast, human activities are drastically affecting the occurrence of golden hamsters in several ways. Hamsters are considered to be the most important agricultural pest besides the vole *Microtus sociales*, which was often found on the same plots. Control measures start in February as soon as the burrow entrances become visible. Animals are

trapped or poisoned. The rural population applies large amounts of rodenticides provided by the government. In May - June most fields are harvested, burnt and ploughed. Sheep herds feed on the remaining plants and grain. At this time it may become increasingly difficult for hamsters to find cover, nutrition or sufficient food for winter storage.

Above all, increasing human settlement caused by an immense population growth of 3.34 % per year provides the main threat to the golden hamster in Syria. However, until now there are only insufficient data to evaluate the abundance and population dynamics of the species and its distribution has not yet been fully clarified.

The captured golden hamsters were brought to the Institute of Zoology in Halle and a breeding stock was set up which has already produced several offspring. Behavioural and genetic studies on potential differences between wild and laboratory hamsters are currently underway and scientific co-operation is welcome. The breeding population of wild golden hamsters in Halle can also be used to enhance the genetic variability of current golden hamster strains.

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4.2. Vergleichende Untersuchungen zur genetischen Variabilität von Labor- und Wildgoldhamstern

Mit dem Aufbau eines neuen Wildstammes von Goldhamstern ergab sich die Möglichkeit einer Reihe von vergleichenden Studien. Die Etablierung eines Markersystems zur Einschätzung der genetischen Variabilität der eingesetzten Zuchtlinien und zur Durchführung von Elternschaftsanalysen war dabei von wesentlicher Bedeutung. **Publikation VI: „Microsatellites for diversity studies in the golden hamster (*Mesocricetus auratus*)“** beschreibt die Isolierung von 10 nukleären Tandemrepeat-Markern. Die Arbeit enthält auch einen ersten Diversitätsvergleich von Labortieren mit Wildfängen aus Syrien. Es konnte gezeigt werden, dass Laborgoldhamster etwa 70 % ihrer genetischen Variabilität im Vergleich zur Wildpopulation verloren haben. Dies liegt allerdings im Bereich anderer, erst in neuerer Zeit etablierter Labortiere, wie z.B. der Mongolischen Wüstenrennmaus (Neumann et al. 2000). Zusätzlich wurde gezeigt, dass sich ein großer Teil der isolierten Mikrosatelliten-Loci auch für genetische Analysen bei den anderen drei *Mesocricetus*-Arten eignet.

Die erfolgreiche Amplifikation einzelner Mikrosatelliten von Gold- und Feldhamster bei anderen Hamstergattungen ermöglichte u. a. die Durchführung einer Studie zum Dispersionsverhalten von *Tscherskia triton* (**“Sex-biased dispersal of greater long-tailed hamster *Tscherskia triton* revealed by microsatellites“)**.

Im Rahmen des Labor-/ Wildhamstervergleichs wurden weitere Arbeiten veröffentlicht. Sie beinhalten Untersuchungen zur Morphologie (**“*Comparative studies of body mass, body measurements and organ weights of wild derived and laboratory golden hamsters Mesocricetus auratus”***) und Spermienkonkurrenz (**“*Differences in the reproductive success between laboratory and wild golden hamsters Mesocricetus auratus as a consequence of inbreeding”*)** der beiden Stämme. Der Vergleich von Körpermaßen und Organgewichten ergab nur marginale Unterschiede zwischen Wild- und Laborhamstern. Dagegen ergab sich ein deutlich höherer reproduktiver Erfolg der Wildhamstermännchen nach erfolgreicher Verpaarung eines Weibchens mit zwei Männchen beider Stämme. Trotz ungeklärter Ursache kann es sich dabei um einen maskierten Inzuchteffekt (Joron und Brakefield 2003; Hoogland 1995) handeln, der im Zusammenhang mit den deutlichen genetischen Unterschieden steht.

4.2.1. Microsatellites for diversity studies in the golden hamster (*Mesocricetus auratus*)

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ABSTRACT

Ten polymorphic microsatellites were developed for the golden hamster (*Mesocricetus auratus*) a widely used model organism in biological and medical research. All loci were used to analyse the microsatellite variability in wild golden hamsters from Syria and in a sample of domestic animals comprising different strains. Average mean expected heterozygosity (HET_{exp}) and mean allele number (A) of domestic hamsters measured 0.279 ± 0.058 and 2.6 ± 0.306 compared to 0.809 ± 0.019 and 8.3 ±1.075 found for wild hamsters. Cross species application in other *Mesocricetus* species proved conservation of most loci throughout the genus.

The golden hamster (*Mesocricetus auratus*) is a widely used laboratory animal (Lowrey et al. 2000, Fritzsche et al. 2000). Its natural distribution area is limited to northern Syria and southeastern Turkey (Gattermann et al. 2001). In 1930, a captive hamster population was established from a single sibling pair (Aharoni 1932) which now increased to several million individuals worldwide. Only a few studies deal with the low genetic variation in laboratory hamsters (McGuire et al. 1985, Watkins et al. 1990) so far. Here we report the development of microsatellites for the golden hamster and present a diversity comparison between laboratory and wild animals.

Isolation of microsatellites followed a modified enrichment protocol by Ostrander et al. (1992) described in Maak et al. (2001). A genomic DNA library was established from six wild hamsters. A total of 768 recombinants were isolated of which 103 appeared positive after a second hybridization with the two chosen microsatellite probes CA/GT and GA/CT. Clones were sequenced with the ALFexpress AutoRead sequencing kit according to the manufacturer and runs were performed on an AL-

Die Markerentwicklung wurde durch mich durchgeführt. Die Fragmentanalysen wurden im eigenen Labor durchgeführt. Die Auswertung der Populationsdaten und die Erstellung des Manuskripts erfolgte durch mich.

Fexpress II DNA analysis system (Amersham Pharmacia Biotech).

Primers were designed for 32 loci using OLIGO 5.1 (MedProbe). After testing, 10 microsatellites were selected for further analyses (Tab. 1). PCR amplification was done in 25µl reactions (puRE Taq Ready-To-Go, mix and go system, Amersham Biosciences) containing ~100 ng genomic DNA and 12.5 pmol of each fluorescently labelled forward and unlabelled reverse primer. After an initial denaturation step of 180 sec. at 94°C, the amplification proceeded for 35 cycles as follows; 60 sec. at 94°C, 60 sec. annealing at primer specific temperatures and 120 sec. at 72°C (Thermocycler UNO II, Biometra). Alleles were separated on an ABI 377 DNA Analyzer and fragment size was determined with GeneScan software and Gene scan 500 [Tamra] length standards (Applied Biosystems).

Table 1 Details of microsatellite loci in *Mesocricetus auratus*

Locus	Primer	Repeat motif	Annealing temp. (°C)	GenBank Accession No
Mau2	F: AAAAGCATAGTAGCGGAGAAA R: CCAAGCAAGCTTTATTTTACAT	$(GT)_{15}$	54	AJ845169
Mau3	F: GCACCAAAGTCTTCCTG R: CCTGAATTAGCTTGTAAAAGT	$(AC)_{18}TC(AC)_7$	48	AJ633818
Mau4	F: ACTTCAGGAAGGAGCATTATT R: TTGGCCAGATAGGGTTTA	$(TG)_{13}$	48	AJ845170
Mau6	F: AAGTAGGGAAGAGTAGAGGAGA R: ACTTTATTTGGTGTTTTCATTTA	$(TG)_{19}T(TG)_3$	50	AJ845171
Mau9	F: AAAAGAAAGGAAAGAAGCAAGAGA R: ATGGGGGAGGGCGGGGAGAA	$(GA)_{33}$	52	AJ845172
Mau10	F: CCCCAATTTCCTTCTTTCA R: ACAACAATTCTCAACCCACAT	$(TC)_{27}$	50	AJ845173
Mau12	F: TTGTGGGGACTGGGAAGA R: AGCATAAACTGTCATTGGTGT	$(AC)_{26}$	50	AJ845174
Mau13	F: TTGAACTAGGGACTCCACTTA R: CAGCCCAATTTTGTTTTCTTTT	$(AC)_{23}$	50	AJ633820
Mau14	F: GAGGTAAGAGTCATTGTCAGGTAA R: ACAATCTCAAAATGCCAGGTTCT	$(GT)_{23}$	54	AJ633819
Mau15	F: ATGCCTGCATCATAGCCTCTTTCA R: AATCCTCAAGTTGCCCTCTGGTGT	$(GT)_{21}$	58	AJ845175

For population comparisons we analysed 18 wild hamsters from three locations around Aleppo/Syria, and 38 laboratory animals comprising three different strains (Charles River/Canada: n=13; Zoh: GOHA Institute of Zoology Halle/Germany: n= 16; Zoh:Goha x Tau mutant SN Toronto/Canada: n = 9). Statistics were mainly computed with GENEPOP ver. 3.4 (Raymond and Rousset 1995). Linkage analysis was

carried out in wild strain pedigrees (4 families, 41 offspring) with the sequential LOD score method. Close linkage could be excluded ($z < -2$ for all $\theta = 0.05$ and $z < 0$ for all $\theta < 0.2$) for all loci except for Mau 2/4, Mau12/13, Mau 14/15 Mau 3/4, Mau 6/4 ($z < 0$ for all $\theta < 0.01$). Evidence for linkage was found for the combination Mau 3/12 ($z = +3.066$ for $\theta = 0.05$). A linkage disequlibrium test combined over all strains (GENEPOP ver. 3.4) also supported linkage between Mau 3 and Mau 12 ($p<0.0001$). Linkage could not be rejected for Mau6/14 and Mau3/12 after sequential Bonferroni correction.

Table 2 Allele numbers, heterozygosity values, tests of Hardy-Weinberg equilibrium (HWE) in *M. auratus* populations and allele diversity of three further *Mesocricetus* species. Loci with asterisks harbour alleles which did not arise from alterations of the predominant repeat motif. Allele length in *italics* refers to sizing difficulties. "?" indicates spurious bands.

Locus	Allele no. Wild *M. au.* Dom *M. au.* (n)	Size range (bp) Wild *M. au.* Dom *M. au.*	HET_{obs} / HET_{exp} Wild *M. au.* Dom *M. au.*	HWE test	Allele no./ size range *M. brandti* *M. newtoni* *M. raddei*
Mau2	8 (18) 1 (38)	128 – 148 142	0.778/0.763 -	0.333/0.021 -	10/142 – 164 2/146 – 160 3/140 – 150
Mau3	6 (18) 3 (38)	84 – 94 88 – 92	0.556/0.775 0.079/0.433	0.088/0.007 <0.001/<0.001	13/58 – 94 4/64 – 90 3/80 – 88
Mau4	5 (16) 3 (38)	155 – 165 155 – 159	0.500/0.724 0.079/0.235	0.089/0.006 <0.001/<0.001	14/167 – 209 6/181 – 205 2/147 – 151
Mau6	7 (18) 1 (38)	202 – 218 216	0.778/0.787 -	0.079/0.009 -	16/184 – 218 2/186 – 188 1/210
Mau9	10 (18) 3 (38)	108 – 151 111 – 145	0.722/0.787 0.211/0.351	0.834/0.020 0.004/0.001	?/? ?/? 3/143 – 161
Mau10*	17 (18) 4 (38)	185 – >300 181 – 207	0.944/0.952 0.297/0.406	0.484/0.040 0.051/0.004	*13/191 – 234* ?/? 2/209 – 217
Mau12*	9 (18) 3 (38)	192 – 210 204 – 210	0.667/0.844 0.105/0.309	0.148/0.015 <0.001/<0.001	16/183 – 213 5/200 – 236 3/220 – 226
Mau13	7 (18) 3 (38)	215 – 227 219 – 225	0.778/0.808 0.368/0.513	0.906/0.006 0.096/0.005	4/215 – 225 2/217 – 219 1/*195*
Mau14	6 (18) 3 (38)	196 – 206 202 – 210	0.778/0.810 0.105/0.127	0.233/0.009 0.011/0.002	9/188 – 236 5/178 – 206 3/212 – 220
Mau15*	8 (18) 2 (38)	165 – 182 170 – 178	0.667/0.835 0.263/0.417	0.110/0.010 0.042/0.001	18/156 – 186 2/149 - 151 3/166 - 170

However, because of the relative low numbers of informative individuals in both tests the results could still be chance. Therefore, we retained all loci in diversity analysis. No measurable frequencies of null alleles were detected. The population comparison in golden hamsters revealed the expected reduction of genetic variability in laboratory animals: mean allele number (*A*) of 2.6 (SE = ± 0.306) and mean heterozygosity (HET_{exp}) of 0.279 ± 0.058. Wild golden hamsters had values of *A* = 8.3 (SE = ± 1.075) and HET_{exp} = 0.809 (SE = ± 0.019). All indices were computed in GENEPOP. Tests for Hardy-Weinberg equilibium (HWE) detected no significant deviations in wild hamsters. The pooled laboratory sample shows strong violations of HWE across polymorphic loci, apart for Mau10 (p = 0.051 ± 0.004) and Mau13 (0.096 ± 0.005), which accounts for some strain heterogeneity. The finding corroborates enzyme studies reporting limited protein polymorphisms in laboratory animals (e.g. Kluge et al. 1995). Microsatellite polymorphisms in wild golden hamsters are comparable to the variation found in other Cricetinae populations (Neumann et al. 2004). Despite low sample size it should be kept in mind that the collecting sites of wild golden hamsters cover almost the entire known range. In this respect, our data describe the microsatellite diversity range of almost the entire species.

Finally, all but one microsatellite (Mau9) allowed the detection of distinguishable polymorphic alleles in three other *Mesocricetus* species (*M. brandti:* n=10; *M. newtoni*: n=3 and *M. raddei*: n=3, Table 2). Mau 3, 13 and 14 proved also polymorphic in a study on the rat-like hamster, *Tscherskia triton*, (Song et al. unpublished).

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4.3. Zur Populationsstruktur von Goldhamster (*Mesocricetus auratus*) und Türkischem Hamster (*Mesocricetus brandti*)

Der Goldhamster besitzt ein extrem kleines Verbreitungsgebiet, welches sich auf Nordsyrien und die Südtürkei beschränkt. Er besiedelt hier vor allem landwirtschaftliche Flächen, da die meisten natürlichen Steppenhabitate durch anthropogene Maßnahmen verloren gegangen sind. Die geografische Einschränkung des Areals, im Norden und Westen die Gebirgsausläufer des Taurus und des nordsyrischen Kalksteins, im Osten der Euphrat und im Süden die syrische Wüste, verleiht der Verbreitung des Goldhamsters einen inselartigen Charakter. Das isolierte Vorkommen und die enge Bindung an bewirtschaftete Flächen stellt für den Hamster eine ernsthafte Bestandsbedrohung dar. Insbesondere das enorme Wachstum der menschlichen Besiedlung in diesem Gebiet (ca. 3,34 % Bevölkerungswachstum pro Jahr in Nordsyrien) und eine abzusehende Intensivierung der Landwirtschaft bedeuten einen enormen Risikofaktor. Ähnlich Probleme führten zu dramatischen Bestandseinbrüchen des Feldhamsters in Europa. Eine bisher unbekannte Größe bildet die Klimaentwicklung. Insbesondere der syrische Raum ist von einer fortschreitenden Trockenheit seit dem Pleistozän betroffen. Ein weiteres Vordringen der Wüste in Richtung Norden könnte das Areal des Goldhamsters weiter einschränken.

Etwas anders gestaltet sich die Situation für den Türkischen Hamster, der das größte Areal aller *Mesocricetus*-Arten besiedelt. Es reicht von Zentral-Anatolien bis in den Westen Irans, einschließlich einer kleinen Enklave im ciskaukasischen Dagestan. *M. brandti* besiedelt teilweise sehr trockene Steppenbereiche und kommt in Höhen bis über 2000 m vor (Demirsoy 1999). Die Art ist im Verbreitungsgebiet relativ häufig und morphometrische sowie Färbungsunterschiede lassen eine größere Anzahl von Unterarten vermuten (Yigit et al. unpubliziert). Zur Biologie der Art existiert kaum Literatur (Lyman and O'Brian 1977; Yigit et al. 1997).

Die **Publikation VII: „Evidence for a species-wide bottleneck in the golden hamster - contrasting population histories in two eastern Mediterranean *Mesocricetus* species"** liefert die erste Untersuchung zur Populationsstruktur und demografischen Entwicklung von *M. auratus* und *M. brandti*. Wie in den bereits erwähnten Studien am Feldhamster wurden sowohl mitochondriale Gene (Kontrollregion, 16SrRNA, Cytochrom b) als auch nukleäre Mikrosatelliten eingesetzt. Die Untersuchung zeigt, dass die Population des Goldhamsters nur wenig strukturiert ist. Eine Ursache dafür ist wahrscheinlich intensiver Genfluss zwischen den besammelten

Populationen in Syrien und der Türkei. Satellitenaufnahmen zeigen das Verbreitungsareal des Goldhamsters als einen kontinuierlichen Flickenteppich von Feldern ohne bedeutende geografische oder urbane Barrieren. Selbst bei einer Art mit geringem Migrationsverhalten, sollte stepping stone dispersal zu ausreichender genetischer Kommunikation zwischen Subpopulationen führen. Die geringe Variabilität mitochondrialer Haplotypen deutet gleichzeitig auf einen Flaschenhals hin, der die gesamte Goldhamsterpopulation umfasste. “Pairwise mismatch“ – Analysen in Kombination mit einer molekularen Uhr ergaben, dass der Goldhamster wahrscheinlich vor 42.300 - 73.300 Jahren (Kalkulation von Cytochrom b-Daten) währen der Weichseleiszeit expandierte. Das Zeitfenster stimmt mit dem Auftauchen des Goldhamsters in Israel vor 40.000 – 75.000 Jahren überein (Tchernov 1968, 1975). Leider fehlen Fossildaten für den syrischen Raum, um die historische Entwicklung der Goldhamsterpopulation nachzuvollziehen. Es ist möglich, dass eine Ausweitung arider Steppengebiete während der letzten Eiszeit zu einer Populationsexpansion führte. Alternativ dazu könnte aber der genannte Zeitraum auch die Ankunft des Goldhamsters im derzeitigen Verbreitungsgebiet darstellen. Die enge Verwandtschaft zwischen dem kaukasischen *M. raddei* und *M. auratus* lässt vermuten, dass die letztere Art weiter nördlich, vielleicht in der Kaukasusregion entstand. Klimatische Veränderungen in der Weichseleiszeit oder/und die Ausbreitung von *M. brandti* könnten ein Abdrängen des Goldhamsters nach Süden bewirkt haben. Der ermittelte Flaschenhals würde dann einem Gründereffekt entsprechen.

Im Gegensatz zum Goldhamster zeigt der Türkische Hamster (*M. brandti*) eine ausgeprägte räumliche Struktur bezüglich der Verteilung mitochondrialer Haplotypen. Die am meisten differenzierte Gruppe kommt am südlichen Rand des Konya-Beckens und entlang der Nordseite des Taurus vor. Eine zweite zentralanatolische Linie existiert nördlich des Konya-Beckens. Sie ist stark strukturiert und war Ausgangspunkt einer Ostexpansion im Verlauf des Pleistozäns, die zur Etablierung der Populationen im Bereich des Van-Sees und der Region Ardahan beitrugen. Die westiranische Population dagegen entstammt einer früheren Migrationswelle. Ein ähnliches Ausbreitungsmuster existiert bei der Felsenmaus *Apodemus mystacinus* in der Türkei (Michaux et al. 2005). Die hohe Diversität zentralanatolischer *M. brandti* unterstreicht die Bedeutung des Gebietes als evolutives Zentrum der Art. Trockenperioden, aber auch Abschnitte mit ausgedehnten Salzfluren und Binnenseen veränderten die Besiedlungsmöglichkeiten zentralanatolischer Becken für *M. brandti* während

des Pleistozäns. Das hat unzweifelhaft zur Differenzierung der Hamsterpopulationen in dieser Region beigetragen. Die anhand von morphologischen Merkmalen postulierte Unterteilung von *M. brandti* in eine zentral- und eine ostanatolische Unterart (Yigit et al. unpubliziert), kann anhand der genetischen Befunde nicht bestätigt werden. Obwohl die ausgeprägte Populationsstruktur des Türkischen Hamsters durch pleistozäne Klimaoszillationen beeinflusst wurde, gibt es keine Anzeichen für massive Arealrücknahmen oder das Aussterben von zahlreichen Teilpopulationen wie beim nördlichen Feldhamster.

4.3.1. Evidence for a species-wide bottleneck in the golden hamster *Mesocricetus auratus* - contrasting population histories in two eastern Mediterranean hamster species

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ABSTRACT

We examined the natural population structure of two Eastern Mediterranean hamster species, the golden hamster *Mesocricetus auratus* and the Turkish hamster *Mesocricetus brandti*. Low mitochondrial variability in golden hamsters from northern Syria and southern Turkey is consistent with a historic bottleneck. Genetic depletion might be the consequence of a founder event which occurred as a result of a range shift during the Late Pleistocene. Population structure in golden hamsters is low, indicating high levels of gene flow due to the absence of natural barriers or extensive human infrastructure. The island-like character of the distribution area and the close association with agricultural sites causes a significant threat to the species survival in the future. Increasing urbanisation and modernized farming practise may further reduce the number of inhabitable sites. In contrast to that, *Mesocricetus brandti* shows a high degree of genetic structuring across its range. Most diverged hamster lineages exist north and south of the Konya-basin as well as in western Iran. Genetic separation followed different expansion waves triggered by climatic oscillations throughout the Pleistocene.

Projekt und Experimente wurden von mir konzipiert. Mikrosatellitenanalysen und Sequenzierungen wurden unter Mithilfe der technischen Assistentinnen G. Becke, B. Gebhardt und S. Jäsert durchgeführt. Sämtliche Auswertungen der Daten erfolgten durch mich. Das Manuskript wurde von mir erstellt.

INTRODUCTION

The golden hamster *Mesocricetus auratus* is one of the most popular small mammals representing a well-established laboratory animal and an attractive household pet (Clark 1987). Laboratory golden hamsters reached scientific significance in various fields of medical and biological research (Lowrey et al. 2000, Wong et al. 2003, Johnston 2003). Above all, the entire domestic hamster stock originated from four siblings (three males and one female) caught in 1930 (Aharoni 1932, Murphy 1985, Gattermann 2000) and provides a striking example for a thriving population despite reduced genetic variation (McGuire et al. 1985, Ellegren et al. 1993, Frankham et al. 2000). Today's captive population counts several million individuals worldwide (Gattermann 2000). No other mammal achieved a similar breeding success from such a narrow gene pool. From MHC studies it been suggested that wild hamsters may also exhibit lower levels of genetic diversity (Watkins et al. 1990). However, a recent survey of wild animals from northern Syria using microsatellites revealed a variability level comparable with other outbred rodent populations (Neumann et al. 2005).

Despite a wealth of biological data about domestic golden hamsters, the species belongs to the least known mammals in the wild. Comments on its field ecology are mainly based on anecdotal descriptions and short-time observations (Aharoni 1932, Murphy 1971, Gattermann 2001). One reason for the lack of field data is the narrow distribution range of *M. auratus* restricted to parts of northern Syria and south-eastern Turkey. Wild animals were only sporadically observed or captured (Kefelioglu and Tichy personal communication) and the species was even considered as gone missing in nature by some authors (e.g. Niethammer 1988). Meanwhile, there are new reports on viable populations in Syria and Turkey (Dogramaci et al. 1994, Yigit et al. 2000, Gattermann et al. 2001) and a first study on the species field behaviour is underway. The golden hamster represents, like most Cricetinae species, a typical steppe animal. Its current distribution area comprises mainly agricultural sites because most fertile steppe has been converted into farmland. Geographical barriers like the Taurus Mountains in the north, the Mediterranean Sea in the west, the Euphrates valley in the east and the southerly located Syrian Desert give the *M. auratus* range an island-like character.

M. auratus is the southernmost species of the *Mesocricetus*-hamsters. The genus harbours four allopatric species which are distributed in south-eastern Europe and the Middle East (Wilson and Reeder 1993, Hamar and Shutowa 1966, Pantelejev

1998). All species inhabit semi-arid steppe or cultivated farmland. Some hamsters like *M. raddei* and *M. brandti* can reach mountain plateaus above 2,000 meters. *Mesocricetus* hamsters comprise one of three major phylogenetic lineages of the Cricetinae which evolved about 8 - 11 MY ago in the upper Miocene (Neumann et al. in press). In contrast to other extant hamster groups there is a complete lack of ideas about the putative ancestry of the genus with respect to the current fossil record. Better established is the eastern Mediterranean origin of *Mesocricetus*. Oldest fossils from the late Miocene and Pliocene, identified as *M. primitivus*, were excavated in Chalkidike (L-Miocene/E-Pliocene; Vasileiadou et al., 2003), Rhodos (upper Pliocene; De Bruijn et. al. 1970) and central Turkey (E-Pliocene; Sevket et al. 1998). The distribution of fossil records roughly coincides with current species' ranges implying that *Mesocricetus* hamsters never experienced large area shifts. *Mesocricetus* fossils were also found in Israel dating from the Late Middle to the Late Pleistocene (Tchernov 1975) which could mark the most southern range extension of the genus.

According to DNA data, the genus *Mesocricetus* is divided into two evolutionary lineages, the *brandti*-group consisting of *M. brandti* and *M. newtoni* and the *auratus*-group comprising *M. auratus* and *M. raddei* (Neumann et al. in press). Both groups probably split during the upper Pliocene about 2.5 - 2.7 MY ago. The *brandti*-lineage shows a longitudinal distribution pattern. *M. brandti* inhabits the widest range of all *Mesocricetus* species stretching from central Anatolia to the western part of Iran. *M. newtoni* occurs only in a small area in the Dobrudscha. The species evolved during the early Pleistocene (~1.7 – 1.8 MY) following a westward expansion of the ancestral *brandti*-type lineage. Putative *M. newtoni*-fossils are known from Turkish Thrace during the middle Pleistocene (Santel and von Königswald 1998). The ranges of the *auratus*-group are vertically structured. *M. raddei* is distributed in the Caucasus and adjacent northern steppe areas, whereas *M. auratus* inhabits the most southern range in northern Syria and Turkey, south-east of the Taurus. Both species areas are well separated by geographical barriers and interrupted by *M. brandti* which occupies also a small enclave in Dagestan. The discontinuous distribution of the *auratus*-lineage accounts for some range displacement, probably during the middle Pleistocene. DNA analyses imply a split of *M. raddei* and *M. auratus* 1.2 - 1.5 MY ago (Neumann et al. in press).

Here we provide the first study on the genetic population structure of the golden hamster across its currently known range and compare it with the more widely

spread Turkish hamster. We also attempt to align molecular and fossil evidence to reconstruct the population history of both species. The results may not only support conservation measures for the golden hamster but contributes to the knowledge about the processes governing population structuring and subsequent speciation in the eastern Mediterranean. In this respect, the study will also complement phylogeographic and population studies on Turkish and middle eastern rodents (Michaux et al. 2004, 2005) and other animals (Cook 1997, Weisrock et al. 2001, Fattorini 2002, Veith et al. 2003, Hrbek et al. 2005).

MATERIAL AND METHODS

Animals

Altogether, we used genetic information of 43 golden hamsters (*Mesocricetus auratus*) for our study. Thirty-six golden hamsters were sampled between 1999 and 2005 in Syria and Turkey. One pregnant female gave birth to five offspring and it was possible to deduce the microsatellite genotypes of the siring male. Skin samples of further three golden hamsters (SMF 82129 - 82131) were provided by the Senckenberg Museum (Frankfurt/Main-Germany). These animals were originally caught in Syria in 1986. Three ordinary laboratory golden hamsters from the strain Charles River/Canada were incorporated as descendents of Aharonis initial catch in 1930 at Aleppo/Syria.

Thirty-two Turkish hamsters (*Mesocricetus brandti*) were collected at ten localities in Turkey and one locality in western Iran. Table 1 informs about sampling localities and the corresponding number of collected individuals for both *Mesocricetus* species. Figure 1 and 2 give details about distribution ranges and the geographical location of sampling sites.

DNA analyses

Genomic DNA isolation from fresh or ethanol fixed materials such as ear, liver, muscle followed a standard protocol supplied with the E.Z.N.A. Tissue DNA Kit II system (peqlab Biotechnologie). DNA from museum specimens was extracted using the Fixed-Tissue genomic DNA Purification system (Promega). Before use, museum tissue samples were rinsed three times in destilled water. Extractions from museum samples were carried out in a separate lab unit under special precautions e.g. the use of filter tips with a separate set of pipettes.

Table 1 Geographic origin and number of individuals used for microsatellite and mtDNA analyses. Abbreviations: CA, central Anatolia; EA, eastern Anatolia.

Species	Country	Geographic location	No of animals tested with microsatellites	No of animals tested with mt genes
M. auratus	*Syria*	Arnaz	5*	4
		Albel	4	4
		Shayk-Riek	2	2
		Azaz	5	5
		Aldaheria	1	1
		Jarablus	-	3
		Aleppo	3	2
	Turkey	Kilis	2	1
		Elbeyli	18	18
		All	40	40
M. brandti	*Turkey*	Konya (CA)	1	1
		Nigde (CA)	-	1
		Yesilköy (CA)	1	1
		Meydan (CA)	1	1
		Corum (CA)	1	1
		Kirsehir (CA)	4	4
		Kayseri (CA)	3	3
		Erzurum (EA)	1	1
		Ardahan (EA)	5	9
		Van (EA)	3	4
		?	1	1
	Iran	Zanjan	4	5
		All	25	32

Three partial mitochondrial DNA (mtDNA) genes; control region (*ctr*), 16SrRNA (*16S*) and cytochrome *b* (*cytb*), were investigated. PCR-amplification, purification and sequencing of DNA followed largely a protocol described in Neumann *et al*. (2004, 2005). In cases where direct sequencing produced ambiguous results, the PCR products were cloned into the pGEM-T-vector system (Promega), and subsequently sequenced using the T7 sequencing kit (Amersham). Eight *M. auratus* microsatellites were used. Details of seven loci; Mau3, 4, 6, 9, 10, 14, 15, were already published (Neumann et al. 2005). Additionally we applied a new locus MauX068 (CA_{17}; F: CCACTTCAGGGCTTCTCTGT, R: AGGAGAGAGATTCTGGGGATT, 54°C annealing temperature) which is located in the 5' UTR of the desmin gene (Ac.: X06807). PCR conditions and allele identification followed Neumann et al. (2005).

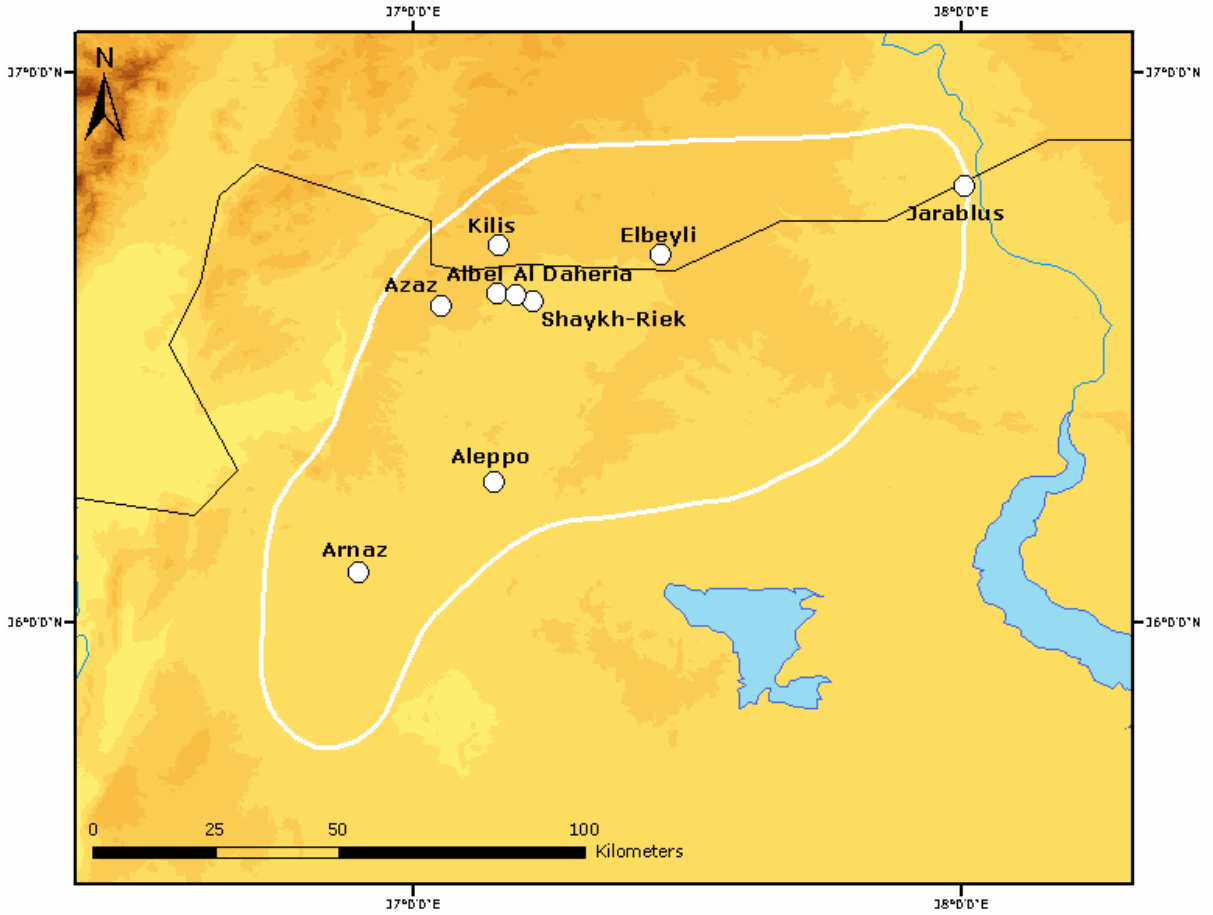

Fig. 1 Geographic distribution of *Mesocricetus auratus* samples. The marked zone refers to the presumed distribution area of the species (modified after Gattermann et al. 2001).

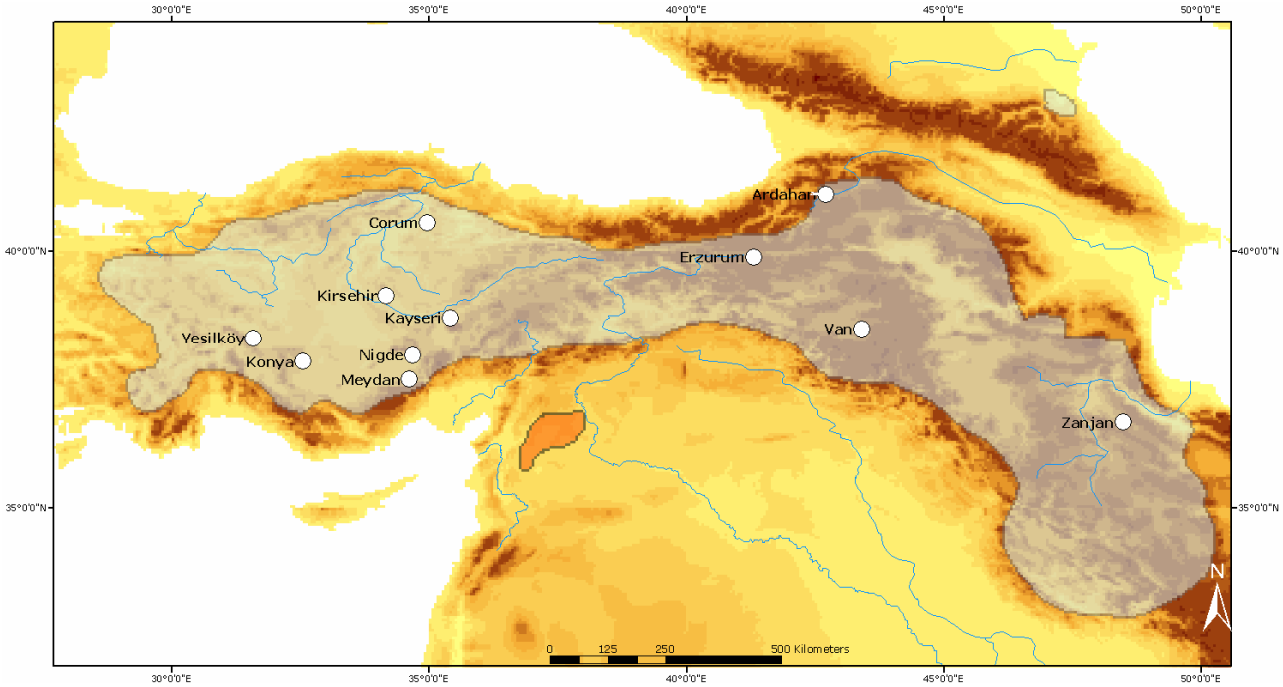

Fig. 2 Geographic distribution of the *Mesocricetus brandti* samples. The shaded zones correspond to the distribution area of the species (partially based on Panteleyev, 1998). The orange area is the *M. auratus* range.

Genetic diversity, population structure and effective population sizes

Mitochondrial DNA

Nucleotide diversity π was calculated in MEGA 2.1 (Kumar et al. 2001) using the Kimura 2-parameter (K2P) distance. Corresponding standard errors were estimated with the bootstrap method (1000 replicates). Haplotype diversity *Hd* was computed in DNASP 4.1 (Rozas and Rozas 1999). An analysis of molecular variance (AMOVA, Excoffier et al. 1992) was conducted to detect structuring in both hamster species. The test was performed in ARLEQUIN 2.001 (Schneider et al. 2000). The maximum-likelihood (ML) method was used to build trees from mtDNA in TREEFINDER (Jobb 2005). We used FINDMODEL (implemented in the web-based HCV data base) to select the maximum-likelihood model which fits best to our data. Median-joining networks were constructed from haplotypes with NETWORK 4101 (Röhl 2000).

The effective population size of females was calculated as $N_{ef} = \pi/2uT$ (Yu et al. 2003, see also Hedrick 2005). We applied a mutation rate of 7.5 – 13 % per million years (MY) as proposed by Galbreath and Cook (2004) for voles. The estimate exceeds other divergence rates used in small mammals e.g. those for *Apodemus*, 2.6 – 2.85 % / MY (Michaux et al. 2005). However, the presumption of a higher mutation rate seems to be more appropriate in population studies regarding the elevated short-term mutation rate (< 1 - 2 MY) of mitochondrial and nuclear genes (Ho et al. 2005). The generation time of *Mesocricetus* hamsters was considered to last six months. Higher reproductive rates, as achieved with laboratory golden hamsters (minimal breeding age is 6 - 8 weeks, Bartke 1985), are very unlikely to occur in nature.

Microsatellites

Mean number of alleles (*A*) and mean heterozygosities H_O and H_e were calculated in GENEPOP v. 3.4 (Raymond and Rousset 1995).

To define potential populations we used the programs STRUCTURE (Pritchard et al. 2001) and BAPS 3.1 (Corander et al. 2003, 2004). Both programs apply a Bayesian method to cluster individual genotypes. STRUCTURE attempts to find the optimal number of clusters (K) under Hardy-Weinberg equilibrium. BAPS 3.1 uses stochastic optimization instead of a Markov Chain Monte Carlo (MCMC) algorithm to create populations which differ by their allele frequencies. An individual-based neighbour-

joining (NJ) tree for the golden hamster was constructed in POPULATIONS (Olivier Langella, CNRS UPR90349) using chord distances (Cavalli-Sforza and Edwards, 1967). Bootstrapping was carried out over the number of loci. The tree was drawn in TREEVIEW (Page 1996).

$N_e = \theta/4uT$, which expresses the effective census size of the entire population, was computed in MISAT 1.0 (Nielsen 1997). The program uses a maximum likelihood approach to find the best θ for a given locus by a markov chain method. Analyses were carried out according to the single-step mutation model. We assumed an average mutation rate of 10^{-4} per generation (Huang et al. 2002) although mutation rates vary considerably between loci and alleles (Ellegren 2000). Only five microsatellites could be included in the analyses. Three loci proved unsuitable because they exhibited allelic variants deriving from different repeat types or alleles with extreme length differences. Since population size estimates are based on frequencies we randomly selected only five individuals (maximum sample from other locations) from the 15 golden hamsters collected in Elbeyli 2005 to reduce sampling bias.

Demographic analysis

Mitochondrial DNA

To detect historic bottlenecks we applied a pairwise mismatch distribution test (Rogers 1995) using ARLEQUIN 2.001 (Schneider et al. 2000). Parametric bootstrapping (1000 replicates) was carried out to test whether obtained mismatch patterns fit a sudden expansion model (Schneider and Excoffier 1999). The same program was used to estimate the expansion parameter τ. Additionally, we applied Fu's *Fs* (Fu 1997), a neutrality statistics which proved to be very sensitive in detecting census size fluctuations (Ramos-Onsins and Rozas 2002). Fu and Li's *F** and *D** (Fu and Li 1993) were calculated (DNASP 4.1) to test whether expansion signals were related to true demographic events or background selection. If only *Fs* is significant and *F** and *D** are not, then population growth or range expansion is indicated, whereas the reverse suggests selection (Fu 1997). Significance of neutrality values was tested by a coalescence simulation in DNASP 4.1. Mutation rates required for expansion time estimates were as described for N_e.

Microsatellites

To detect recent negative size fluctuations we used the program BOTTLENECK

(Cornuet and Luitkart 1996). The test compares observed and expected allele frequencies, taking a significant excess of heterozygotes as indication for a bottleneck.

RESULTS

Table 2 summarizes all diversity values and demographic parameters estimated for the two *Mesocricetus* species.

Genetic diversity in M. auratus and M. brandti

Mitochondrial genes were obtained from 40 *M. auratus* individuals including one domestic animal as a descendant of the historic catch near Aleppo in 1930. Analyses were carried out with all 32 *M. brandti* specimens. A 380 bp segment containing the 5' peripheral domain of the control region was highly similar among four different *Mesocricetus* species (data not shown) and was therefore considered to represent a true mt copy. Only five *ctr* haplotypes were found in *M. auratus* (7 variable sites, 5 singletons, all transitions). One unique haplotype showed three singletons. Nucleotide diversity π and haplotype diversity *Hd* measured 0.339 ± 0.179 % and 0.595 ± 0.008, respectively. *M. brandti* proved much more variable with 21 haplotypes (24 variable sites, 8 singletons, 2 transversions) and larger diversity indices (π = 1.594 ± 0.365 %, *Hd* = 0.933 ± 0.006). Nine *cytb* haplotypes (925 bp; 9 variable sites, all singletons, all transitions) could be identified in *M. auratus*. One is identical to the sequence (AF119265) published by Conroy and Cook (1999) proving that we obtained true mt sequences. Three mutations caused amino acid exchanges. Nucleotide diversity π was 0.119 ± 0.048 % and *Hd* measured 0.753 ± 0.008. Twenty-three *cytb* haplotypes were present in the *M. brandti* sample (134 variable sites, 17 singletons, 8 transversions). Only nine substitutions led to amino acid changes. Nucleotide diversity π was 4.417 ± 0.417 %. *Hd* measured 0.956 ± 0.004. PCR of *16S* led to the amplifcation of more than one gene copy in *M. auratus*. One was invariant among all specimen (472 bp analyzed) and supposedly the real mtDNA copy. A second copy showed a number of allelic morphs due to length variations of a short $T_{(n)}$ cluster. Cloned sequences of both *16S* types had a similarity of ~95 % (8 TV/15 TS). However, because of the uncertainty about the origin of both copies we excluded the *M. auratus 16S* gene from statistical analyses. Fourteen *16S* haplotypes (473 bp) occurred in *M. brandti* (35 variable sites, 6 singletons, 2 transversions). Nucleotide di-

versity π was 1.396 ± 0.31 % and *Hd* numbered 0.837 ± 0.01.

Microsatellite typing was performed with 36 wild *M. auratus*. Genotypes of an additional individual were obtained by analyzing a complete litter and deducing the microsatellite alleles of the missing father. Unfortunately, we failed to produce reliable genotypes from the museum specimens. Number of alleles range from 5 (Mau4) to 20 (Mau10). Mean allele number measured 10.750 ± 1.916. H_o and H_e were 0.710 ± 0.068 and 0.785 ± 0.052, respectively. Microsatellites were amplified in a maximum of 25 *M. brandti* individuals. Some individuals exhibited additional bands which did not allow faithful genotyping. Microsatellite diversity is high with A = 17.14 ± 0.99, H_o = 0.79 ± 0.04. Expected heterozygosity was not calculated because of the heterogeneous sampling.

Table 2 Mitochondrial haplotype (haplotype number N_H, nucleotide diversity π, haplotype diversity *Hd*) and microsatellite diversity indices (allele number A, observed heterozygosity H_o, expected heterozygosity H_e) of the golden (*M. auratus*) and the Turkish hamster (*M. brandti*).

	Mitochondrial genes			**Microsatellite loci**
Hamster species	N_H (n), *ctr*, *cytb*, *16S*, *ctr+cytb* *ctr+cytb+16S*	π ± SE	*Hd* ± SE	A ± SE / n H_o ± SE / n H_e ± SE / n
M. auratus	5 (40)	0.339 ± 0.179	0.595 ± 0.008	10.750 ± 1.916 / 37
	9 (40)	0.119 ± 0.048	0.753 ± 0.008	0.710 ± 0.068 / 37
	- (40)	-	-	0.785 ± 0.052 / 37
	13 (40)	0.183 ± 0.066	0.860 ± 0.005	
M. brandti	21 (32)	1.594 ± 0.121	0.933 ± 0.006	17.143 ± 0.986 / 25
	23 (32)	4.417 ± 0.417	0.956 ± 0.004	0.789 ± 0.038 / 25
	13 (32)	1.396 ± 0.310	0.837 ± 0.010	-
	25 (32)	3.561 ± 0.317	0.966 ± 0.010	-
	25 (32)	3.209 ± 0.222	0.966 ± 0.010	-

Population structure and effective census sizes

No clear regional partitioning was achieved with mt haplotypes or microsatellite allele distributions for *M. auratus*. The median-joining network of combined *ctr+cytb* is dominated by a star-like cluster of haplotypes which harbours sequences of all sampled localities (Figure 3). However, most diverged haplotypes occur in the most southerly located Arnaz (Syria) and AMOVA gives evidence for a weak structure when grouping individuals according to the most distant locations (Arnaz, Jarablus, all others): *Fst* = 0.190, p = 0.024. But the two groups of Arnaz (n = 4) and Jarablus (n = 3) are very small which may inflate the outcome of the test (81 % of variation within and 19 % of variation among populations).

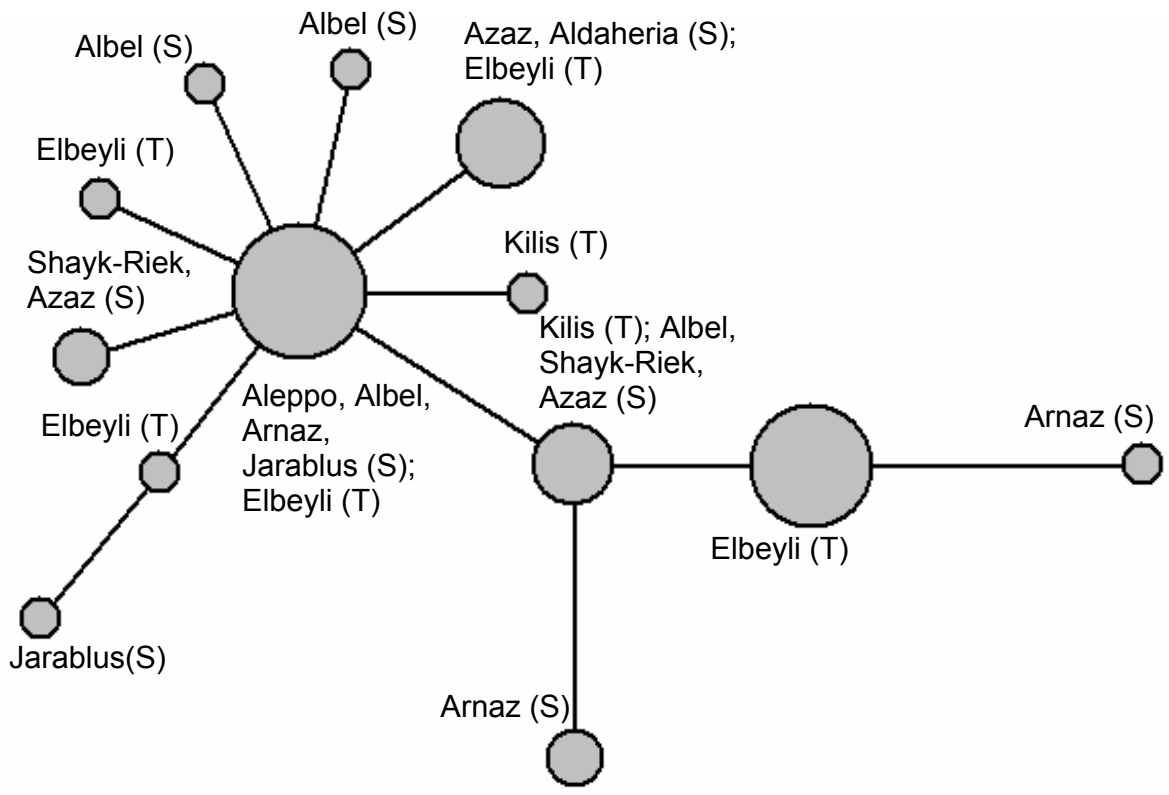

Fig. 3 Median-joining network of combined *ctr+cytb* haplotypes of 40 golden hamsters (*Mesocricetus auratus*). The location Aleppo is a laboratory animal which represents a descendant of the initial catch in 1930. Numbers are given in cases where haplotypes are divided by more than one mutational event.

In contrast, the median-joining network (Figure 4) and the NJ gene tree (Figure 5) revealed extensive population structuring in *M. brandti*. For network constructions we combined only *cytb* and *ctr* sequences because the large number of mutated sites when using all three genes prevented the calculation of a single network. Gene trees were based on all three mtDNA segments to obtain maximal resolution. Tree building was performed with the HKY+Γ+I algorithm. The HKY-model (Hasegawa et al. 1985) appeared the most appropriate for *cytb* sequences and was among the top three models selected for *ctr+cytb+16S* combined. Altogether, three main clades could be defined. Animals from the Konya-region in the southern part of central Anatolia (Konya, Meydan, Nigde, Yesilköy and one animal of unknown origin) proved most diverged from all other Turkish hamsters (D_{acomb} = 4.82, 95%CI: 3.95 – 5.68; D_{acytb} = 8.13, 95%CI: 6.23 – 10.03). A second group is formed by animals from western Iran which are separated from the rest of the Anatolian population (D_{acomb} = 2.44, 95%CI: 1.71 – 3.16; D_{acytb} = 3.27, 95%CI: 2.21 – 4.32).

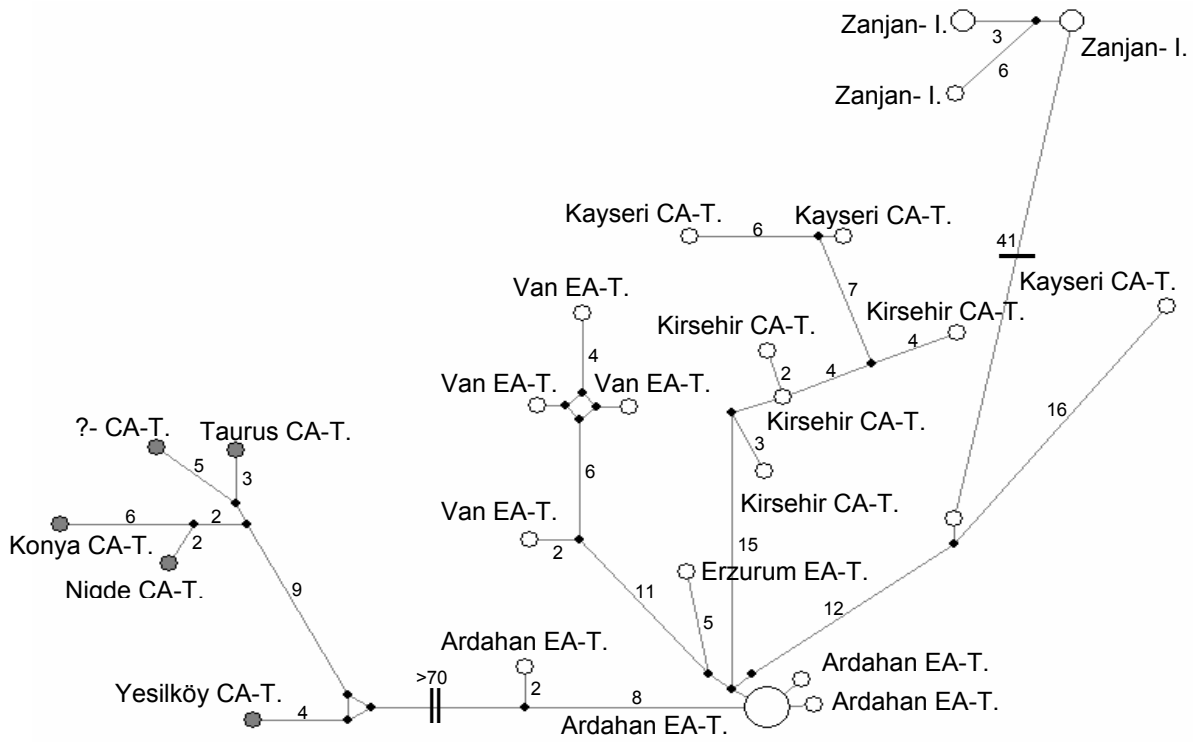

Fig. 4 Median-joining network of *ctr+cytb* haplotypes obtained from 32 Turkish hamsters (*Mesocricetus brandti*). Hamsters were sampled in Turkey (T.) and western Iran (I.). Numbers on links refer to mutational steps dividing haplotypes. Dark diamonds comprise missing haplotypes. Abbrevations: CA-central Anatolia, EA-eastern Anatolia.

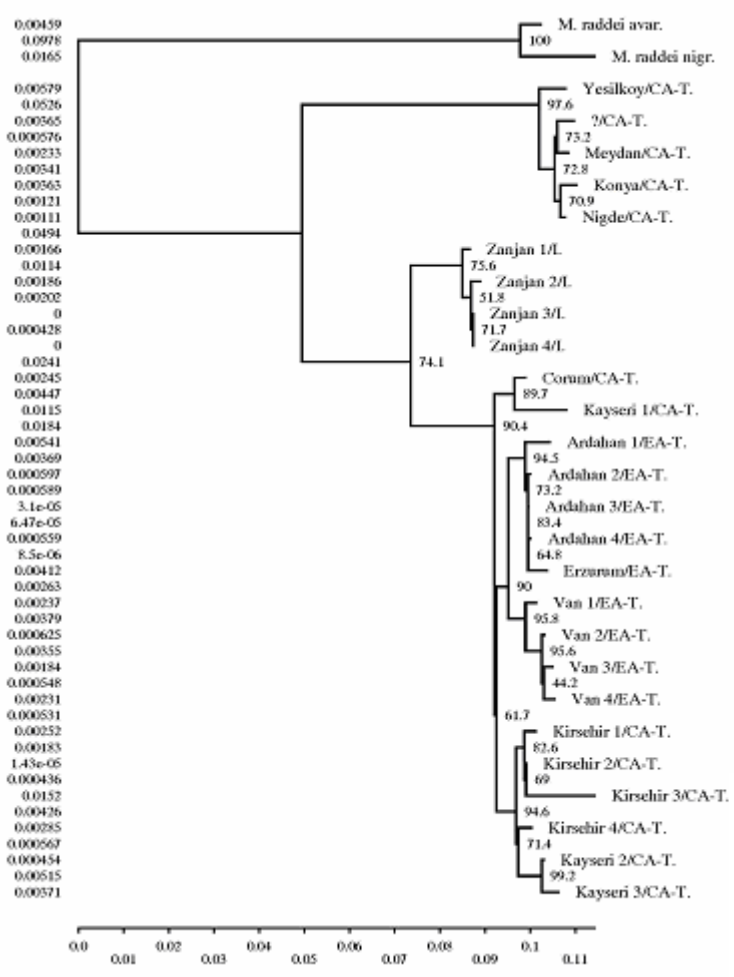

Fig. 5 Neighbour-joining tree of *M. brandti* haplotypes (*ctr+cytb+16S*) using the HKY+Γ+I model of evolution. Numbers on branches are bootstrap values based on 1000 replicates. Abbreviations are as in Fig. 3.

The third lineage of *M. brandti* includes animals from central Anatolia (north of the Konya-basin) and hamsters from eastern Anatolia. This lineage shows further significant sub-structuring. Separation of groups is supported by high bootstrap values in gene trees. Structure in *M. brandti* was also confirmed by AMOVA based on subdivision (F_{ST} = 0.798, p > 0.001) as suggested by network and gene tree data. About 80 % of all mtDNA variation was found among populations and only 20 % within populations. Population structure analyses using microsatellites produced no clear spatial clustering in *M. auratus*. STRUCTURE (burn in period: 10,000; MCMC: 10,000; repeated 3 times) was run under the presumptions of an admixture model. We have chosen the admixture option because of the expected high proportion of alleles with common ancestry due to the close proximity of most collecting sites. BAPS also failed to produce a consistent structure under mixture and admixture conditions. Most proposed clusters contained individuals from various localities. The NJ tree (Figure 6 constructed from 40 individual microsatellite genotypes (37 wild, 3 laboratory hamsters), appears star-like with low bootstrap values and shows also no signs of a clear regional structure.

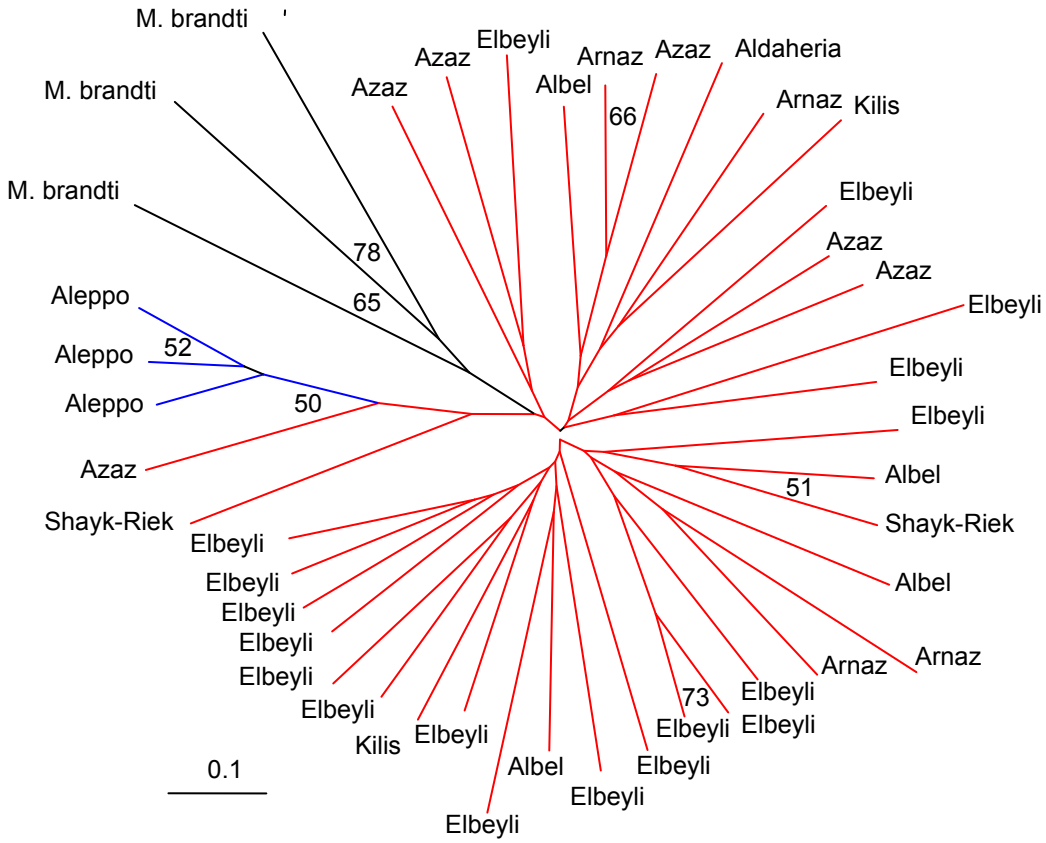

Fig. 6 Unrooted NJ tree based on individual microsatellite frequencies of 40 golden hamsters *Mesocricetus auratus*. Samples from Aleppo are laboratory hamsters. Three Turkish hamsters *Mesocricetus brandti* serve as outgroups. Bootstrapping was carried out over the number of loci.

N_{ef} was estimated from combined *ctr*+*cytb* haplotypes to be ~31,000 - 53,000 individuals (95%CI: ~5,900 - 141,300). N_e measured 35,900 animals but large differences of θ among loci (4.65 - 43.18) led to a relatively large confidence interval of 95%CI: 59 - 71,600 hamsters limiting the validity of the estimate. Similar to the previous species, we obtained no faithful structuring with microsatellites in *M. brandti*. A likely reason for that is the high number of different microsatellite alleles in the sample which lead to many unique genotypes. Only five individuals from Ardahan/Turkey consistently clustered. Four of them were caught at the same spot and they may actually comprise relatives.

Demographic parameters

Pairwise mismatch data and neutrality tests gave inconsistent results for *ctr* and *cytb* in both hamster species (Table 3; Figures 7, 8).

For *ctr* haplotypes a p-value of 0.059 did not reject expansion of the *M. auratus* population, but a positive *Fs* = 0.355 (p = 0.612) was in favor of a stationary population. *F** (-1.61) and *D** (-1.72) were not significant (p > 0.05). In case of *cytb* we obtained a significant value (p= 0.046) for the mismatch distribution, which rejects expansion. In contrast, the *Fs* value for *cytb* is significantly negative with -4.143 (p = 0.008). Non-significant *F** and *D**, -1.44 (p = 0.11) and -1.15 (p = 0.12) respectively, support expansion. For the combined *ctr*+*cytb* we obtained a significant expansion signal (p = 0.354 for mismatch distribution, *Fs* = -4.425, p = 0.024; *F** = 1.80, p = 0.08; *D** = 1.70, p = 0.07). Neither mismatch distributions nor Fu's *Fs* can faithfully rule out a sudden population growth or range increase scenario for the golden hamster population. Therefore, we calculated expansion times from the following Tau (τ) values; *ctr* = 2.436 (95%: 0.308 - 5.785), *cytb* = 1.268 (95%: 0.193 - 1.842), *ctr*+*cytb* = 3.017 (95%: 1.138 - 5.683). Expansion times for *ctr*, *cytb* and *ctr*+*cytb* were 198 - 343 ky (95%: 12.5 – 407.4 ky), 42.3 - 73.3 ky (95%: 6.4 - 106.4 ky), and 71.2 - 123.6 ky (95%: 26.8 - 232.9 ky), respectively. There is no evidence for a very recent bottleneck in *M. auratus* according to microsatellite data. A two-tailed Wilcoxon test proved to be not significant assuming infinite allele mutation-drift equilibrium (IAM, p = 0.250) or a stepwise mutation modus (SMM, p = 0.383). Mismatch data revealed a striking difference between mtDNA genes in *M. brandti*.

Table 3 Parameters τ and *Fs* calculated from mitochondrial genes. Estimated expansion times are given in thousands of years (ky).

Hamster species	mt Locus	ratio: singletons/ parsimonious sites	τ (95%CI) / *Fs*	Expansion times in ky
M. auratus	*ctr*	5/2	2.436 (0.308-5.785), p=0.059 / +0.355, p=0.612	198 - 343 (12.5 - 407.4)
	cytb	9/0	1.268 (0.193-1.842), p=0.046 / - 4.143, p=0.006	42.3 - 73.3 (6.4 - 106.4)
	ctr+cytb	2/14	3.017 (1.138-5.683), p=0.354 / - 4.425, p=0.024	71.2-123.6 (26.8 - 232.9)
M. brandti	*ctr*	8/16	- , p=0.747/ -10.197, p=0.002	-
	cytb	17/117	- , not calc./ +0.645 , p=0.626	-
	16S	6/29	- , p=0.677/ -1.402 , p=0.361	-

There is a strong expansion signal in the *ctr* with a clear unimodal curve shape (p = 0.747) and *Fs* = -10.197, p = 0.002 (Figure 8A). There is no evidence for selection (*F** = -0.405, p = 0.331; *D** = -0.402, p = 0.316). In contrast *cytb* and *16S* support a stable population size (Figure 8B, C), *cytb*: *Fs* = 0.645 (p = 0.626), *F** = 1.005 (p = 0.895), *D** = 1.068 (p = 0.934); *16S*: mismatch distribution p = 0.677, *Fs* = -0.658 (p = 0.361), *F** = 0.05 (p = 0.521), *D** = 0.042 (p = 0.498). A p-value for the *cytb* mismatch curve could not be calculated because of the large number of mutations dividing haplotypes. No significant negative *Fs* values were obtained for any population cluster when calculated from combined mt gene haplotypes.

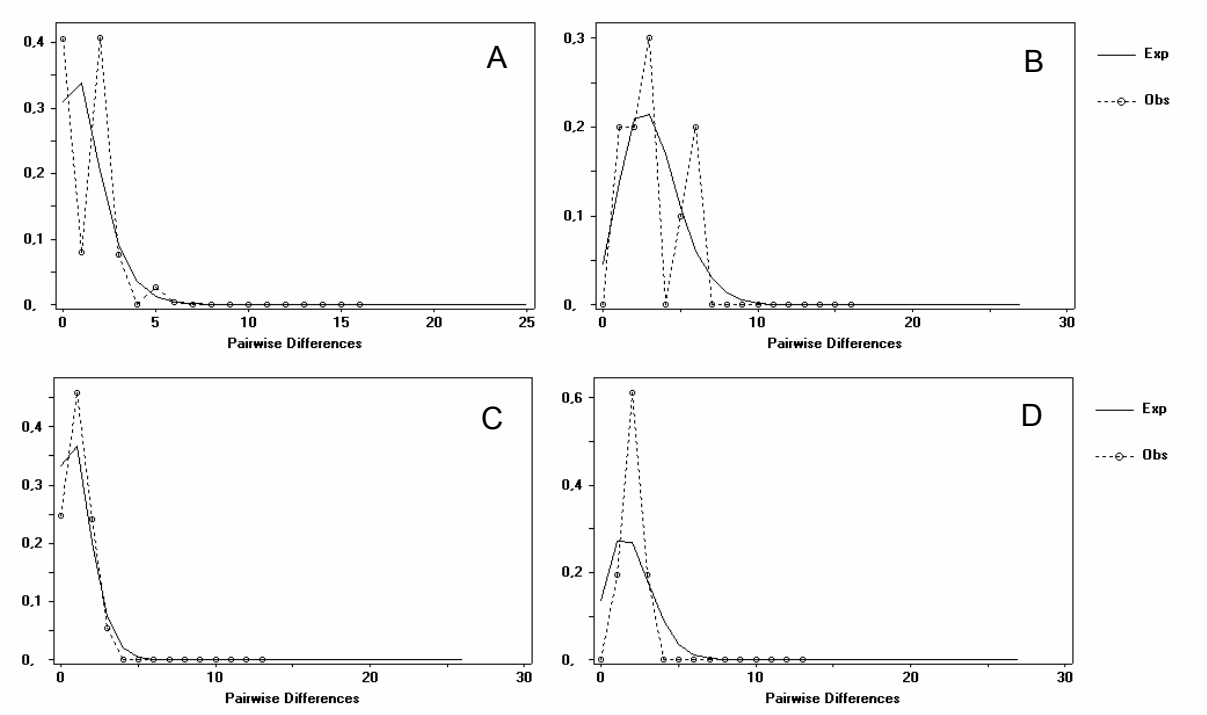

Fig. 7 Pairwise mismatch curves of *ctr* haplotypes (A: total sample, n = 40; B: different haplotypes, n = 5) and *cytb* haplotypes (C: total sample, n = 40; D: different haplotypes, n = 9) of *M. auratus*.

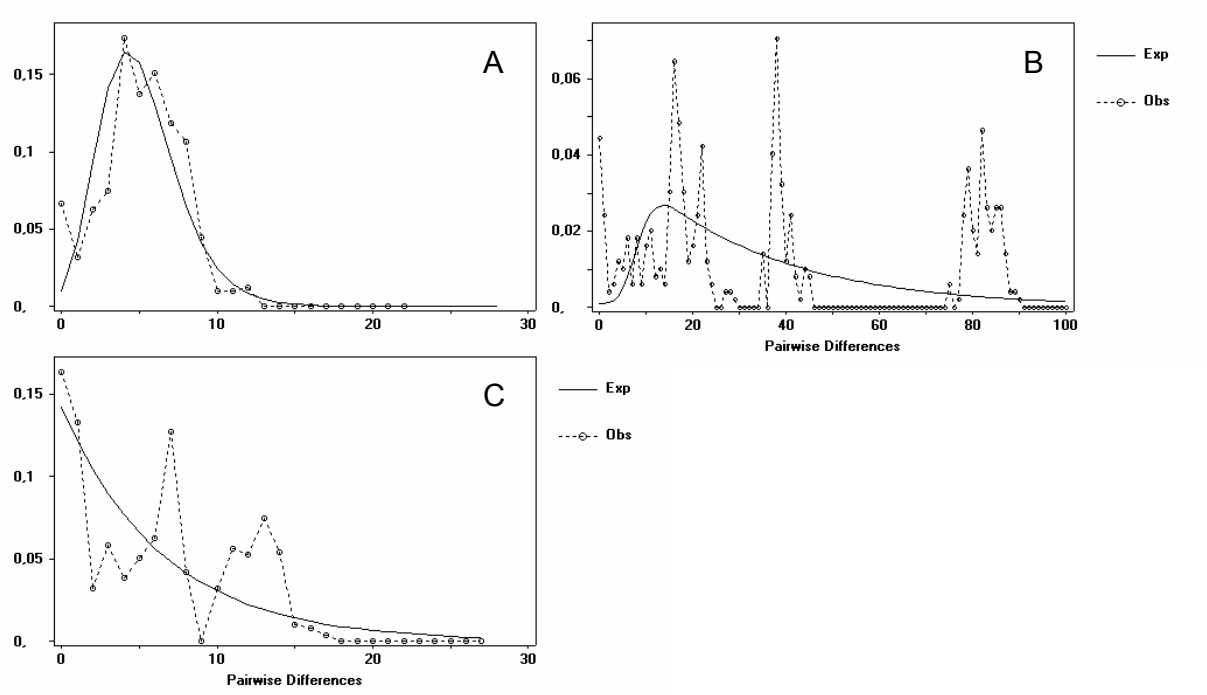

Fig. 8 Pairwise mismatch curves of *ctr* (A), *cytb* (C) and *16S* haplotypes of *M. brandti* (total sample).

DISCUSSION

Diversity and population structures in East Mediterranean hamsters

Mitochondrial gene variability proved to be very small in the golden hamster *Mesocricetus auratus*. The most likely reason for that is a historic bottleneck because microsatellite heterozygosity analysis does not support a recent population decline. In contrast to mtDNA results, microsatellite diversity is high and similar to other outbred hamster populations (Neumann et al. 2004, 2005). The finding does not cause true contradiction because nuclear microsatellites show a much higher effective population size and therefore, are less affected by bottlenecks (Zhang and Hewitt 2003). Furthermore, higher mutation rates in neutral microsatellites may lead to a quick restoration of genetic variability after a crash. More surprising is the obvious lack of a clear spatial structure in the contemporary golden hamster population despite the fact that most distant sampling sites (e.g. Arnaz – Kilis) are more than a hundred kilometers apart. Dispersal in hamsters is supposed to be low and may depend on population denities (Calinescu 1931, Wang et al. 1996, Weinholdt 1998, Song et al. 2005). Limited migration and population size fluctuations can lead to a quick fragmentation of hamster populations, a phenomenon which is also typical for orther related rodent species (Gerlach and Musolf 2000, Berthier et al. 2005, Neumann et al. 2005, Xie and Zhang 2005). However, the golden hamster area consti-

tutes mainly a patchwork of small fields without extensive infrastructure, vegetation barriers (e.g woods) or geographic obstructions. Stepping stone dispersal could have efficiently counteracted spatial structuring in the past and may still play a significant role for gene flow.

So far, there are no reliable estimates of the actual population size of the existing wild golden hamster population. Gattermann (2000) calculated the current hamster population in Syria of about 50,000 – 200,000 individuals. This is a very crude figure based on questionnaires and sporadic burrow counts. In Turkey, the species is considered to be very rare (Demirsoy 1996, Yigit et al. 2000) and its exact distribution was not yet thoroughly investigated. Population sizes of hamster species can be very high in particular on agricultural fields with abundant food supply. Common hamster *Cricetus cricetus* densities in central German provinces peaked in the late 1960's with more than a million animals captured per year (Stubbe et al. 1998). The area roughly compares to the size of the golden hamsters range. In that respect, the above mentioned figure may represent a relative conservative estimate for the total population size of the golden hamster. Our calculated effective census sizes of ~31,000 - 53,000 (female population size) and ~36,000 (general population size) are in the range of the field estimates because effective population sizes could well be about a magnitude or even lower than the actual census size (Bartley 1992, Frankham 1995).

The situation for the Turkish hamster *M. brandti* is completely different from that of the previous species, mtDNA and microsatellite diversities are much higher than in *M. auratus*. Divergence among haplotype groups in *M. brandti* also exceeds measures of other hamster populations occupying a much wider geographical area e.g. *Cricetus cricetus* (Neumann et al. 2005). However, K2P distances of *cytb* haplotypes of 0.11 ± 0.11 % - 10.88 ± 1.2 % are in the range of those reported for other small mammal species (Cosson et al. 2005). Surprising is the low nucleotide diversity and haplotype distance of the non-coding *ctr* compared to the *cytb* gene (assuming that we consider true mt genes). The *cytb* net distance separating the most diverged haplotype group in the Konya-Taurus region from all other *M. brandti* measured 8.13 % (95%CI: 6.23 – 10.03 %) whereas the *ctr* distance was only 0.96 % (95%CI: 0.10 – 1.82 %). In this respect, the substitution patterns of the two genes are also significantly different from those in *M. auratus* (Fishers exact test, p = 0.009). Such dis-

crepancies between *cytb* and *ctr* DNA were not observed in other rodent studies (Galbreath and Cook 2004, Neumann et al. 2005). Although the low number of transversions in both *ctr* and *cytb* haplotypes does not imply saturation, median joining networks indicate some homoplasy affecting the two genes. But no sites with multiple substitutions were detected in *ctr* sequences (0/*ctr*, 3/*cytb*, 0/*16S*). Selection is also an unlikely factor because we obtained no significant *F** and *D** values for any of the mtDNA genes. An alternative explanation are slight differences in the substitution rates among genes over time. It could be shown, that mtDNA coding regions evolve more clock-like than the *ctr*, but the mechanism behind that is not yet understood (Ingman et al. 2000, Howell et al. 2004). Furthermore, Ho et al. (2005) demonstrated that the short-term mutation rates in *cytb* and *ctr* genes are substantially higher than their actual long-term substitution rates (see also Penny 2005). It is possible that rate effects become more pronounced when comparing evolutionary young (or recently bottlenecked) population with evolutionary old and relatively stable populations. However, although elevated short-term change rates can explain higher *ctr* diversity in the recently bottlenecked *M. auratus* population it can not be the cause for the unexpected low diversity within the *ctr* of *M. brandti*. Unusual low divergence among *ctr* haplotypes (~1 %) were also found in other studies for instance between different *Apodemus agrarius* subspecies (Koh et al. 2000).

Although, the relatively low sample size does no allow a very detailed population analysis, there is clear evidence for substantial genetic differentiation among Turkish hamster populations. Most distinguished groups are found in central Anatolia suggesting that the evolutionary centre of the species lies here. This is also supported by reports of an additional rare *M. brandti* karyotype of n = 44 in inner Turkey, compared to the typical diploid chromosome number of n = 42 (Popescu and Di Paolo 1972, Lyman and O'Brien 1977). Most diverged hamsters occur in the Konya region and along the northern part of the Taurus Mountains. Separation times for the Konya-Taurus lineage of 0.37– 0.64 my (CI95%: 0.30 – 0.76%) and 0.63 - 1.08 (0.48 – 1.34%) for *ctr+cytb+16S* and *cytb* respectively correspond well to Pleistocene climatic changes affecting the Konya basin (Erol 1978, Karabiyikoğlu et al. 1999). Following the desiccation of the extensive Pliocene lake system in central Anatolia, alternating periods of dryness and of spreading lakes with marshy steppe caused dramatic oscillations in the living conditions within the Konya basin during the Pleistocene. Deteriorating ecological conditions may have pushed hamsters repeatedly to

the south and perhaps interrupted a corridor to the north along the foothills of the Taurus. Today, the Konya basin represents a highly arid steppe area with extreme conditions even for xeric steppe animals. However, further sampling may allow the identification of contact zones between the southern lineage and hamsters north of the basin. Distinct eastern populations in western Iran and eastern Turkey (Ardahan and Van) indicate repeated range expansions in the course of the upper and late Pleistocene. Iranian hamsters may have split from the Anatolian population about 251.5 – 436 ky (CI95%: 170 – 576 ky) ago. A fossil record for *Mesocricetus* from western Iran dates to the slightly younger Mousterian period (Turnbull 1975) but it is well possible that *M. brandti* reached the area much earlier. The geographic pattern of *M. brandti* in Anatolia largely matches that of the broad-toothed field mouse *Apodemus mystacinus* (Michaux et al. 2005). Mice from north-western Turkey form a single clade with eastern Anatolian individuals. This indicates the existence of a migration corridor across the northern half of Turkey connecting to the Caucasus and the Iranian plateau. Unfortunately, the presence of a highly differentiated southwestern lineage of *A. mystacinus* in Turkey is not congruent to our findings. The population exists south of the Taurus and has certainly originated from a different geographic or climatic event than the Konya-Taurus lineage in *M. brandti*. A limited survey of *Apodemus flavicollis* covering Turkish animals failed to produce a significant spatial structure. Reason for that is a recent spread of this wood species following the last glacial maximum (Michaux et al. 2004). That the apparent spatial structure of mtDNA genes in *M. brandti* was not confirmed with microsatellites may lie in the highly polymorphic nature of the latter. Microsatellites mutate very quickly, creating a large number of alleles (Jarne and Lagoda 1996, Ellegren 2000). Hence, sampling of single (or too few) individuals from various sites may lead to a heterogeneous collection of different genotypes obscuring the underlying population group structure. Finally, it should be mentioned that the genetic structure in *M. brandti* conflicts systematic analyses using morphometrical and fur colour pattern. External and cranial measures have been recently used to establish subspecies ranks for different local populations (Yigit et al. unpublished). For example, grey coloured morphs are only found in central Anatolia whereas pale and dark morphs occur exclusively in eastern Anatolia. Whether these colour morphs reflect in fact local adaptations remains to be investigated.

Historical demography

Most Hamsters are r-strategists which rapidly increase in numbers under favorable environmental conditions. On the other hand, populations reach equally fast equilibrium with extensive sub structuring. In this respect, it might be difficult to detect past population or range expansion in particular when applying gene frequency based statistics assuming panmixis such as commonly used pairwise mismatch distributions and Fu's *Fs* (Marjoram and Donnelly 1994, Ray et al. 2003, Excoffier 2004). Furthermore, most statistics used to detect a past bottleneck is dependent on mutation events after the crash and may become less reliable when only a few mutations have occurred since then (for discussions on the power of neutrality tests see Galtier et al. 2000, Ramos-Onsins and Rozas 2002). In case of the golden hamster the controversy among mismatch and neutrality tests and among genes cannot easily be explained but both statistical tests are clearly hampered by the low haplotype variability. It is also obvious that the hypothesized bottleneck had different consequences on *ctr* and *cytb* genes. There are two main *ctr* haplotypes surviving in the population causing the bimodal curve pattern (Fig. 7B) which deviates from the expected unimodal shape under the assumption of a population recovery scenario. A severe bottleneck can further lead to the dominance of a single haplotype (or a few) which results in a smooth curve pattern also typical for a population at equilibrium (Rogers and Harpending 1992). In our case, *ctr* and *cytb* pairwise mismatch curves shift towards the y-axis because of the dominance of one or two haplotypes (Figure 7). The high number of singletons dividing different haplotypes is also in favor of a population growth scenario. Bottlenecks typically shorten genealogies depleting the number of parsimonious sites in a gene tree. Subsequent expansion will in contrast enhance the number of unique mutations. This is exactly what is seen in the golden hamster. Altogether, we found 14 singletons but only two parsimonious sites (*ctr*: 2/5, *cytb*: 0/9) which most likely comprise mutation events after the population crash. If the reduction in mtDNA diversity is caused by a historic bottleneck two main questions arise. When did the bottleneck occur and what caused the reduction in population size? Our molecular clock data from combined *ctr*+*cytb* suggest an expansion of the golden hamster population 71.2 - 123.6 ky (95%: 26.8 - 232.9 ky) ago. Unfortunately, there are no fossil records from the investigated area to further pinpoint that estimate. However, Tchernov (1975) examined *Mesocricetus* fossils from the Levante and concluded that *M. auratus* appeared in Israel during the upper Levalloiso-Mousterian

period, about 40 – 70 ky ago and probably earlier. A second species *M. aramaeus* was also found in Palaestine at least since 120 ky ago (Tchernov 1975) and first *Mesocricetus* hamsters appeared in Israel probably more than 250 ky ago. Our time estimates fall therefore in line with historic appearances of the golden hamster and related species in the Middle East. Furthermore, expansion times based on *cytb* alone 42.3 - 73.3 ky (95%CI: 6.4 - 106.4 ky) closely fit the fossil dating from Israel. According to Tchernov (1975) increasing xeric steppe habitats supported the spread of *Mesocricetus* in Syria during the Weichselian. More difficult to find is an explanation for the proposed bottleneck. One possibility is increasing competition with other Cricetids during times of more mesic steppe conditions. Such a factor was stressed by Tchernov (1975) to explain the rapid increase in *Mesocricetus* fossils and the equally rapid disappearance of larger hamsters in the upper Levalloiso-Mousterian layers. Another potential cause arises from the close phylogenetic relationship of *M. auratus* and the Caucasian *M. raddei* (Neumann et al. in press). It is well possible that *M. auratus* evolved further north of its current distribution area and that a range shift caused the species bottleneck. Low mtDNA variation could then be the result of a low number of founders which established themselves in the new environment of southern Turkey and northern Syria. From there *M. auratus* expanded south into Israel until increasing desertification pushed the species back to its contemporary range at the beginning of the Holocene. Unfortunately, there are again no reliable fossil data available to test the range shift-hypothesis. In particular the common use of the species name *M. auratus* for *M. brandti* hampers the alignment of molecular data and fossils from western Asia (e.g. Vereshagin 1958). Michaux et al. (2005) found that broad-toothed field mice (*Apodemus mystacinus*) from north-western and eastern Turkey form a phylogeographic cluster with animals from Syria and Georgia. This provides evidence for a connection between the Caucasus and Syria during the Quarternary, although the ecological demands of the two species are quite different (Mitchell-Jones 1999). It also remains an open question why *M. auratus* evaded to the south. One possibility is tectonic changes and instabilities during the Pleistocene, e.g. a further uplift of the Taurus (Lüttig and Steffens 1975). Effects of tectonic changes on biogeographical pattern of different vertebrates in Turkey have already been reported (Veith et al. 2003, Hrbek et al. 2004) although most these events predate our divergence estimates. An intriguing alternative could be that *M. brandti* displaced *M. auratus* in the course of an eastward expansion.

A highly multi-modal mismatch distribution pattern of *cytb* haplotypes in *M. brandti* is consistent with a long-term persistence of the species in wide parts of its range. Evidence for that comes from a number of Pleistocene fossil records, although reports do not always distinguish between *M. auratus* and *M. brandti* (Vereshchagin 1959, Güleç et al. 1999, Koufos 2001). If we presume that *16S* mutates slower than *cytb* (Pesole 1999) both patterns may nicely reflect the progress of population disintegration in *M. brandti* (Figure 8). Most surprising, the *ctr* mismatch curve suggests a completely different scenario. The unimodal pattern is concordant with a recent population expansion which is supported by a significantly negative *Fs*-value. Possible explanations for the contradicting behaviour of the tested mtDNA genes, e.g. homoplastic mutations, were already discussed above. However, the *ctr* of *M. brandti* has a much lower ratio of singletons versus segregating sites (8/16) than *M. auratus* (5/2) what is expected for a population with a constant size. The entire Turkish hamster population persisted in central Anatolia probably since the late Pliocene/early Pleistocene. From there the population expanded in several waves to the east throughout the Pleistocene establishing populations in Iran and eastern Anatolia. It is possible that such range increases were more phalanx like than leptokurtic (Ibrahim et al. 1996, Hewitt 2000) what could explain the absence of bottleneck signals in hamsters from eastern Turkey. However, a more quantitative sampling is required to answer that question. Although current distribution maps imply a relatively continuous range (Demirsoy 1996, Pantelejev 1999) Turkish hamsters may in fact comprise of long-term isolates with varying levels of divergence. This is concordant with the theory of a predominantly heterogeneous population structure in Mediterranean small mammals (Bilton et al. 1998). Climatic fluctuations in Turkey lead to the spread of populations as well as to a high degree of allopatry but never caused large scale extinction as seen in central and northern Europe. This relatively stable phylogeographic pattern in the past is reflected by the contemporary rich small mammal fauna in Turkey.

Implications for conservation

Mescocricetus auratus has an international conservation status (IUCN Red List of Threatened species, 2004) but no real protection plans exist so far. The species occupies a very narrow distribution range and *M. auratus* is strongly bound to agricultural sites since natural steppe as an alternative habitat has almost completely disappeared in the area. As a consequence, the situation for the golden hamster proves

ambiguous. On one hand, the species certainly benefits from the abundant food supply which has a positive influence on population densities. On the other hand, the close association with farming bears the danger that changes in the agricultural system may have quick and direct affects on the population. An intensive farming system is presumed to be the major reason for the dramatic collapse of the meanwhile critically endangered common hamster populations in western and central Europe (Backbier et al. 1998, Kayser and Stubbe 2003). Fortunately, the annual application of large scale rodentizids in Syria and Turkey has not yet endangered the population. A longterm effective population size of a minimum of 30.000 individuals exceeds by far the critical population size defined by the IUCN for a threathened population. The domestic golden hamster lineage already proved the species potential to recover even from a minimal population size (at least under safe captive conditions). However, most threathening for the species is its narrow and isolated distribution area. A fast growing human population and the increasing demand for modernisation and infrastructure may endanger the golden hamster in the near future. Therefore it is advisable to develop protection measures and managment plans in time, integrating the needs of the local farmers.

M. brandti is still widespread in Turkey and the species is not as tightly linked to human activities. Further studies are required to infer its subspecies problematic and to define potential 'distinct population segments' (US Fish and Wildlife Service & National Marine Fisheries Services 1996, see also Michaux et al. 2005). Completely unclear is the situation of the species in western Iran where the pecies is probably only locally abundant. However, the species is listed in the red data book of Georgia and considered as endangered by the increasing desertification of the Causcasus.

Conclusions

Both Middle Eastern hamster species investigated show completely different population structures, diversity and demographic histories. Whereas *M. brandti* exhibits extensive structuring across its range, the entire species of *M. auratus* is limited to an island – like population with only a minor spatial pattern. Pleistocene climatic events are likely to have triggered migration and population divergence in *M. brandti*. In the case of *M. auratus* a range shift during the Weichselian may have involved the entire species and low mt diversity could therefore be the result of a founder event. The strong association of human agriculture and the occurrence of *M. auratus* will provide

a major challenge for conservationists to work out protection measures in the near future. Noteworthy, is the apparent discrepancy in haplotype diversity and pairwise mismatch analyses between non-coding *ctr* and coding *cytb* genes in both species. The result may arise from variances in the clock-like behaviour among genes as well as differences in substitution rates between species due to different population histories. Furthermore, the result provides an example that conclusions based on single genes could be misleading.

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5. Zusammenfassung und Ausblick

Die hier präsentierten Publikationen liefern grundlegende Ergebnisse zur Populationsstruktur und Systematik paläarktischer Hamsterarten. Es wurde die erste molekulare Systematik der Cricetinae vorgelegt, die einer Reihe von bisher vertretenen taxonomischen Auffassungen deutlich widerspricht. Sie bildet den Grundstock für die weitere Revision der Cricetinae und liefert daneben eine wichtige Orientierung für die Neubewertung von Fossilfunden. Neben neuen Einsichten in die phylogenetische Verwandtschaft bekannter Hamsterarten und Gattungen zeigt sich aber auch die Notwendigkeit einer umfangreichen Überarbeitung der Zwerghamstergattung *Cricetulus*, die sich als nicht-monophyletisch erwies. Die geringen genetischen Abstände zwischen *Cricetulus griseus* und *C. barabensis* (Cytochrom b: ~ 4 %) deuten, entsprechend dem genetischen Artkonzept (Bradley und Baker 2001), wohl eher auf eine komplexe Unterartenstruktur der *barabensis*-Gruppe. Nachfolgende Untersuchungen sollten bisher wenig bekannte und sehr umstrittene Arten, wie z.B. *Cansumys canus*, *Cricetulus kamensis* und *Cricetulus alticola* beinhalten. Eine erste 12SrRNA Analyse von *Cricetulus* (*Urocricetus*) *lama* stellt diesen überraschenderweise in die Nähe von *Phodopus* (Lebedjev et al. 2003).

Einsichten in den Einfluss vergangener klimatischer Prozesse auf die räumliche Struktur von Populationen bilden eine wichtige Grundlage für das Verständnis von Differenzierungs- und damit auch Artbildungsprozessen. Pleistozäne Klimaschwankungen haben unterschiedliche Signaturen in der genetischen Variabilität und Diversität der untersuchten Hamsterarten hinterlassen. Während *M. brandti* eine ausgeprägte räumliche Verteilung mit stark genetisch differenzierten Subpopulationen aufweist, zeigt der Goldhamster kaum Anzeichen einer deutlichen Populationsstruktur. Interessanterweise ist der Feldhamster, der das bei weitem größte Areal besiedelt, zwar geografisch gegliedert, doch zeigen “pairwise mismatch“-Analysen der Cytochrom b-Haplotypen einen unimodalen Kurvenverlauf (Abb. 1). Dieser Befund könnte daraufhin deuten, dass er sich erst in jüngerer Zeit ausbreitete.

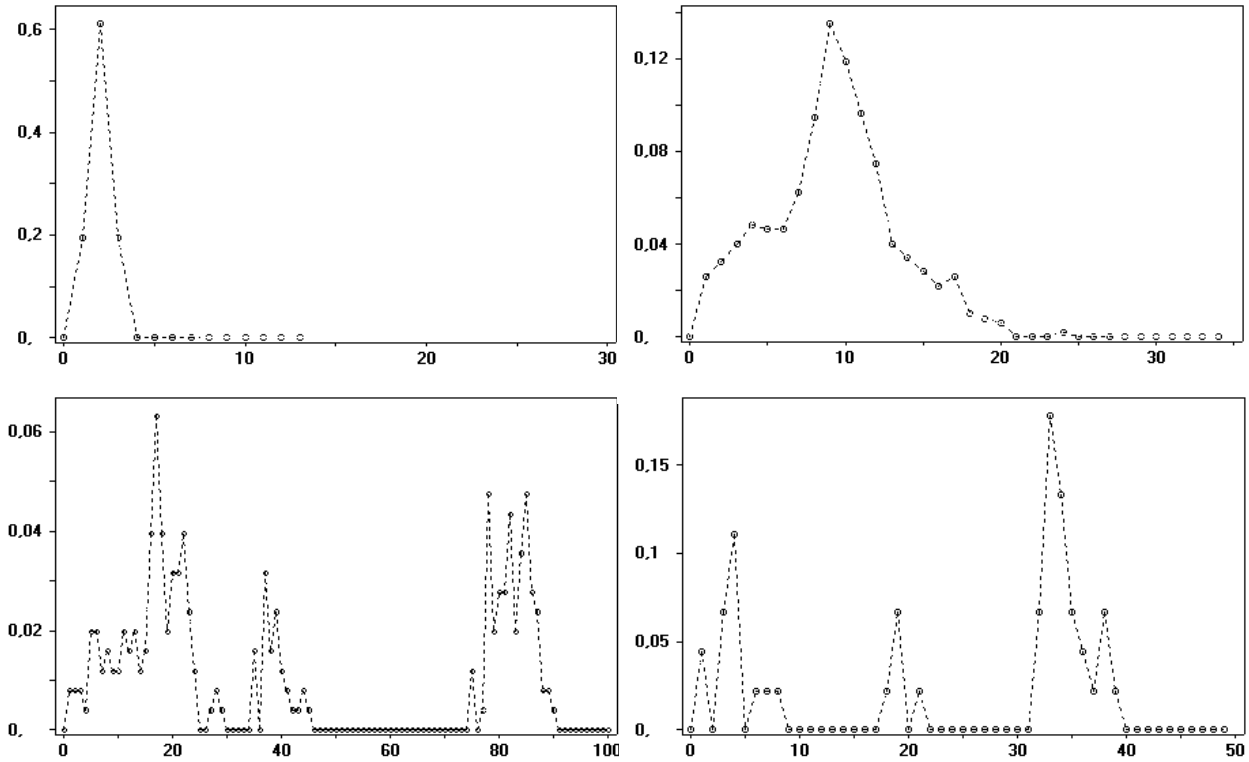

Abb. 1 "Pairwise mismatch" – Kurven der Cytochrom b - Haplotypen von vier Hamsterarten. Links oben: Goldhamster *Mesocricetus auratus*, rechts oben: Feldhamster *Cricetus cricetus*, links unten: *Mesocricetus brandti*, rechts unten: Streifenhamster *Cricetulus barabensis* - Gruppe. Y-Achse: Frequenz, x-Achse: Nukleotiddifferenz.

Feld- und Goldhamster sind stark an landwirtschaftliche Flächen gebunden und weisen eine größere Abhängigkeit von anthropogenen Einflüssen als *M. brandti* auf. Besonders die westliche Feldhamsterpopulation und der Goldhamster zeigen einige Gemeinsamkeiten. Beide Populationen kommen an Verbreitungsgrenzen der jeweiligen Art oder Gattung vor, welches ihre Sensitivität gegenüber ökologischen Schwankungen erhöht. Tatsächlich könnte auch eine fortschreitende Klimaerwärmung für den kontinuierlichen Rückgang der westlichen Feldhamsterpopulationen mit verantwortlich sein (feuchtere Winter, frühere Ernten). Neue Studien belegen nicht nur aktuelle, klimabedingte Arealveränderungen für eine Reihe von Organismen, sondern sagen teilweise dramatische Verschiebungen von Verbreitungsgebieten für die nächsten Jahrzehnte voraus (Gian-Reto et al. 2002; Erasmus et al. 2002; Root et al. 2003). Randpopulationen sind dabei natürlicherweise früher betroffen, als Populationen in Verbreitungszentren. Nach Thomas et al. (2004) sind ca. 15 % aller Steppenarten durch Klima bedingten Habitatverlust bedroht. Inselpopulationen wie die des Goldhamsters könnten besonders betroffen sein, im speziellen Fall durch die fortschreitende Trockenheit im Levanteraum. Obwohl der diskutierte Einfluss aktueller

Klimaentwicklungen auf Populationsprozesse der diskutierten Hamster noch spekulativen Charakter besitzt, sollte dieser unbedingt in weitere Untersuchungen zur Bestandsentwicklung des Feldhamsters in Europa einbezogen werden.

Feld- und Goldhamster besitzen eine verringerte genetische Variabilität, die zumindest im Falle einiger westlicher Subpopulationen des Feldhamsters (z. B. Niederlande) zu einer Reduktion der Fitness geführt haben könnte. Ursachen für reduzierte Polymorphismen sind wahrscheinlich bei beiden Hamsterarten Gründereffekte bedingt durch rasche Arealerweiterung. Dabei entsprechen westliche Feldhamster eher dem Modell einer vorauseilenden Population, während beim Goldhamster möglicherweise die gesamte Population betroffen war. Um dies zu klären, sind aber weitere Untersuchungen zur Fossilienlage und zur Genetik pleistozäner Hamster erforderlich. Die vorgelegten Populationsstrukturen von Feldhamster *Cricetus cricetus*, Goldhamster *Mesocricetus auratus* und Türkischen Hamster *Mesocricetus brandti* ergänzen wesentlich die Theorien zu Verbreitungsmustern anderer Kleinsäuger in Europa und dem westlichen Asien, da Steppenarten bisher kaum untersucht wurden. Mit der Identifizierung evolutionärer Linien und genetischer Gemeinsamkeiten zwischen fragmentierten Populationen beim Feldhamster erfüllen die Arbeiten auch die Anforderungen einer modernen Artenschutzkonzeption.

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7. Habilitationsrelevante Publikationen

Schwerpunktpublikationen

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II **Neumann K**, Jansman H (2004) Polymorphic microsatellites for the analysis of endangered common hamster populations (*Cricetus cricetus* L.). *Conservation Genetics*, **5**, 127-130.

III **Neumann K**, Jansman H, Kayser A, Maak S, Gattermann R (2004) Multiple bottlenecks in threatened western European populations of the common hamster *Cricetus cricetus* (L.) *Conservation Genetics*, **5**, 181-193.

IV **Neumann K**, Michaux JR, Maak S, Jansman HAH, Kayser A, Mundt G, Gattermann R (2005) Genetic spatial structure of European common hamsters (*Cricetus cricetus*) – a result of repeated range expansion and demographic bottlenecks. *Molecular Ecology*, **14**, 1473-1483.

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VI **Neumann K,** Maak S, Fritzsche P, Gattermann R (2005) Microsatellites for diversity studies in the golden hamster (*Mesocricetus auratus*). *Molecular Ecology Notes*, **5**, 876-878.

VII **Neumann K,** Maak S, Fritzsche P**,** Yigit N, Colak E**,** Gattermann R (Manuskript) Evidence for a species-wide bottleneck in the golden hamster - contrasting population histories in two eastern Mediterranean *Mesocricetus* species.

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8. Anhänge

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Hiermit erkläre ich an Eides statt, dass die Habilitationsschrift selbständig und ohne fremde Hilfe verfasst wurde. Andere als die angegebenen Quellen und Hilfsmittel wurden nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche kenntlich gemacht.

Halle, den 07. 12. 2005

Karsten Neumann