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Comparative cytogenetics of hamsters of the genus *Calomyscus*

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Abstract. Karyotypes of *Calomyscus* from different regions of Turkmenistan, Iran, and Azerbaijan were studied using chromosome banding (G- and C-banding) and analyses of meiosis in laboratory hybrids. Extensive variation in the diploid number and the number of autosomal arms (FNa) was revealed (2n = 30, FNa = 44; 2n = 32, FNa = 42; 2n = 44, FNa = 46; 2n = 44, FNa = 58; 2n = 37, FNa = 44; 2n = 50, FNa = 50; 2n = 52, FNa = 56). Centric and tandem fusions and heterochromatin changes were identified as the major modes of karyotype evolution in this group. Natural hybrids between individuals with different karyotypes were recorded, and regular chromosome pairing in meiosis was observed in laboratory hybrids. Fluorescence in situ hybridization with a 353-bp *Bsp*RI complex tandem repeat indicated that chromosomal repatterning occurred recently within the genus. There is no unequivocal evidence suggesting the role of chromosomal change in the speciation of the populations of *Calomyscus* examined.

The genus *Calomyscus* was long considered monotypic and represented by the species *C. bailwardi* distributed in Iran (the type locality in the Khuzistan province), Afghanistan, Pakistan, Turkmenistan, and the southern part of Azerbaijan-Naxçivan (Ellerman and Morrison-Scott, 1951). However, Vorontsov et al. (1979) treated most of the subspecies of *C. bailwardi* as distinct species, and Musser and Carleton (1993) recognized five species, including *C. bailwardi, C. hotsoni, C. mystax, C. tsolovi,* and *C. urartensis.*

Four distinct karyotypes of *Calomyscus* have been reported from the former USSR. Two of these, *C. urartensis* from Naxçivan (2n = 32) (Matthey, 1961; Radjabli, 1975; Graphodatsky et al., 1989) and *C. mystax* from northwestern Turkmenistan (2n = 44, FNa = 46) (Graphodatsky et al., 1989), were recognized as distinct species by Vorontsov et al. (1979) and Musser and Carleton (1993). The taxonomic status of the populations from central and eastern Kopetdag (Turkmenistan), which had a similar chromosome number to *C. mystax* (2n = 44) but differed in the number of autosomal arms (FNa = 58) (Graphodatsky et al., 1989), and the populations from western and central Kopetdag with 2n = 30 (Vorontsov et al., 1979; Graphodatsky et al., 1989) is not yet clear.

In 1997, three new karyotypes, possibly representing chromosome races and/or undescribed species, were collected in

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Table 1. Locality, gender, and karyotype of the 34 hamsters examined

Locality	Longitude, latitude	Region, country	Specimens	Species/race (2N/FNa)
1 Dzhulfa	45°39′ E, 38°57′ N	Naxçivan, Azerbaijan	2 M, 1 F	*Calomyscus urartensis* karyotype 1 (32/42)
2 Great Balkhan Mountains	54°31′ E, 39°26′ N	Balkhan Region, Turkmenistan	2 M	*Calomyscus mystax* karyotype 2 (44/46)
3 Ersary Ridge	55°04′ E, 40°47′ N	Balkhan Region, Turkmenistan	1 M	*Calomyscus mystax* karyotype 2 (44/46)
4 Fir'uza (14 km SW of Ashgabat)	58°05′ E, 37°55′ N	Akal Region, central Kopetdag, Turkmenistan	4 M, 1 F	Karyotype 3 (44/58)
5 Chuli (36 km W of Ashgabat)	58°04′E, 38°02′ N	Akal Region, central Kopetdag, Turkmenistan	1 M	Karyotype 3 (44/58)
6 Kalininsk	58°50′ E, 37°45′ N	Akal Region, central Kopetdag, Turkmenistan	1 F	Karyotype 3 (44/58)
7 Almadjik military point, 5 km S of Bakharden	57°26′ E, 38°23′ N	Akal Region, central Kopetdag, Turkmenistan	1 M	Karyotype 3 (44/58)
8 G'aurs (36 km E of Ashgabat)	58°43′ E, 37°49′ N	Akal Region, eastern Kopetdag, Turkmenistan	1 M	Karyotype 3 (44/58)
9 Archenyan military point	59°37′ E, 37°21′ N	Akal Region, eastern Kopetdag, Turkmenistan	1 M	Karyotype 3 (44/58)
10 Akar-Chashme military point	61°15′ E, 35°55′ N	Mary Region, western Badkhyz, Turkmenistan	1 M	Karyotype 3 (44/58)
11 Agh Mazar Abad (valley 20 km W of Kapkan)	58°32′ E, 37°22′ N	Kopetdag, Khorassan Province, Iran	2 M, 1 F	Karyotype 3 (44/58)
12 Summit of Mt. Dushak	58°10′ E, 37°50′ N	Akal Region, central Kopetdag, Turkmenistan	2 M	Karyotype 4 (30/44)
13 Chaek, 4 km SE from site No. 12	58°10′ E, 37°50′ N	Akal Region, central Kopetdag, Turkmenistan	1 M	Karyotype 4 (30/44)
14 Ai-Dere	56°30′ E, 38°20′ N	Balkhan Region, western Kopetdag, Turkmenistan	1 M	Karyotype 4 (30/44)
15 Gyzylarbat	56°17′ E, 38°58′ N	Balkhan Region, western Kopetdag, Turkmenistan	1 M	Karyotype 4 (30/44)
16 Danata	55°40′ E, 39°10′ N	Balkhan Region, western Kopetdag, Turkmenistan	1 M	Karyotype 4 (30/44)
17 Little Balkhan Mountains	54°53′ E, 39°07′ N	Balkhan Region, Turkmenistan	1 F	Karyotype 4 (30/44)
18 Golubinnaya Schel, Mt. Dushak, N slopes	58°10′ E, 37°50′ N	Akal Region, central Kopetdag, Turkmenistan	2 M	Natural hybrids, karyotypes 3 and 4
19 Vicinity of Karambast and Khassanabad	46°30′ E, 34°58′ N	Bahtaran Province, Iran	1 M	Karyotype 5 (37/44)
20 Shah-Dad Tunnel	57°30′ E, 30°06′ N	Kerman Province, Iran	1 F	Karyotype 6 (52/56)
21 Sivand	52°55′ E, 30°08′ N	Zagros Mountains, Fars Province, Iran	2 F	Karyotype 7 (50/50)
22 Tehran, lower station of the Telecabin Road	51°25′ E, 35°42′ N	Tehran Province, Iran	1 M	Karyotype 2 (44/46)

Iran and are herein reported and reviewed relative to those from other studied populations. In order to clarify the phylogenetic relationships between the known species and chromosome races, we carried out a comparative G- and C-banding analysis and we examined the chromosome location of a 353-bp *Bsp*RI tandem repeat. The implications of this analysis were tested in the study of chromosome pairing in meiosis in laboratory hybrids between parents with different karyotypes.

Materials and methods

Animals studied

The 34 specimens examined were collected from free-living populations at 22 localities in Azerbaijan, Turkmenistan, and Iran (Table 1 and Fig. 1). Skins and skulls of the specimens are deposited in collections maintained by the Zoological Institute, Russian Academy of Sciences, in St. Petersburg and the Department of Zoology, Charles University, in Prague.

Hybrids were obtained in the laboratory from the following parental combinations: *C. urartensis* × karyotype 4 (2 M); *C. urartensis* × karyotype 3 (2 M); *C. mystax* × karyotype 3 (2 M); karyotype 3 × karyotype 4 (3 M).

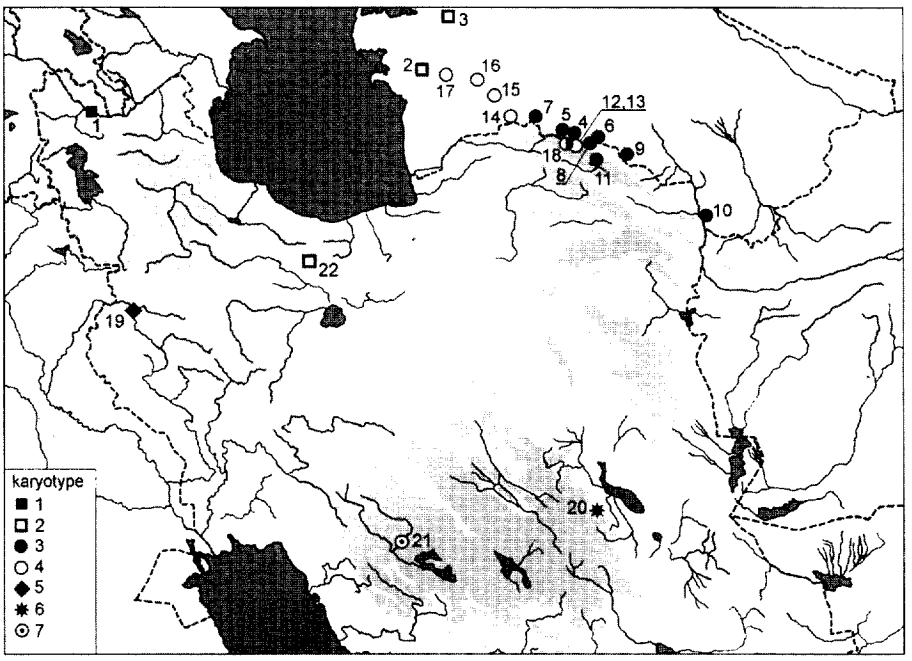

Fig. 1 . Geographic position of the localities studied in the region between the Caspian Sea and the Persian Gulf. For details on each map position, see Table 1.

Chromosome analysis

Metaphase chromosomes were prepared from bone marrow cells by conventional procedures. G-banding was performed according to Seabright (1971), as modified by Graphodatsky and Radjabli (1988). C-band staining was performed after Sumner (1972). Electron microscopic analysis of synaptonemal complexes (SCs) was performed in *C. mystax* × karyotype 3, and *C. mystax* × karyotype 4 male hybrids. Surface spreads of primary spermatocytes were prepared from testes of adult male laboratory hybrids according to the method described by Chandley (1989) and stained with silver nitrate (Howell and Black. 1980). At least 30 cells of each male were examined and photographed with a JEM-1250 electron microscope (JEOL) at 75 kV.

Fluorescence in situ hybridization (FISH)

Genomic DNA was prepared from fresh liver by phenol-detergent extraction (Henry et al., 1990) and digested to completion with *Sau*3A and/or *Bsp*RI. Electrophoresis of DNA was performed in 4% polyacrylamide gel. Repeated sequences of the 350-bp *Bsp*RI fragment were isolated from individuals with the karyotype 3 and cloned in pUC19. The clones were subsequently analyzed using the method described by Mayorov et al. (1996). DNA blot hybridization was performed by standard methods at 63 °C, and single-strand DNA for the sequence analysis was obtained according to Sambrook et al. (1989). Commercial restriction endonucleases were obtained from Fermentas (Vilnius, Lithuania) and SibEnzyme (Novosibirsk, Russia).

This clone was used for FISH in chromosomes of *C. urartensis*, *C. mystax*, and individuals with the karyotypes 3, 4, and 5, using the technique described by Pinkel et al. (1986). The probes were denatured at 65 °C for 10 min and then preannealed by incubation at 42 °C for 15–60 min. Slides were denatured by incubation in 70% formamide, 2 × SSC solution at 65 °C for 1.5–2 min, then quenched in ice-cold 70% ethanol and dehydrated through a 70%, 90%, and 100% ethanol series. The preannealed paints were applied on slides, covered with a 22 × 22 mm^2 cover slip, and incubated overnight at 42 °C. Post-hybridization washes were three 3-min incubations in 50% formamide, 50% 2 × SSC at 45 °C, followed by one 5-min incubation in 2 × SSC and two 3-min incubations in 0.2 × SSC at 45 °C. FITC-labeled probes were visualized using rabbit-anti FITC (1:200) and goat-anti-rabbit FITC antibodies (1:100). After detection, the chromosomes were stained with DAPI (2 mg/ml) and mounted in Antifade AF1 (Citifluor).

Results

Karyotypes

Karyotype 1 (C. urartensis). The karyotype possessed 32 chromosomes (Figs. 2A and 3A), including six pairs of biarmed autosomes and nine pairs of acrocentrics of decreasing size (FNa = 42). Some of the acrocentrics showed secondary constrictions. The autosomes and the X chromosome contained small C-heterochromatin blocks at the centromeres. The Y chromosome was completely heterochromatic. The complement was recorded in populations from the Naxçivan region in Azerbaijan.

Karyotype 2 (C. mystax). The karyotype contained 44 chromosomes (Figs. 2B and 3B), including two pairs of subtelocentric autosomes and 19 pairs of acrocentric autosomes (FNa = 46). The short arms of the subtelocentric autosomes were C-band positive. The X chromosome contained a heterochromatic short arm. The Y chromosome was completely heterochromatic. The karyotype was found in the Great Balkhan Mountains in Turkmenistan and in a single male from Tehran, Iran.

Karyotype 3. The karyotype comprised 44 chromosomes (Figs. 2C and 3C). Usually, there were eight pairs of subtelocentric autosomes and 14 pairs of acrocentric autosomes (FNa = 58). The subtelocentric autosomes possessed heterochromatic arms, and their number varied in different populations from seven to nine. The X chromosome was an intermediate-sized submetacentric with a heterochromatic short arm. The Y chromosome was completely heterochromatic. The karyotype was recorded in central and eastern Kopetdag and western Badkhyz in Turkmenistan and in Khorassan Province in Iran.

Karyotype 4. The karyotype contained 30 chromosomes (Figs. 2D and 3D), including eight pairs of large biarmed autosomes and six pairs of small acrocentrics (FNa = 44). Almost all

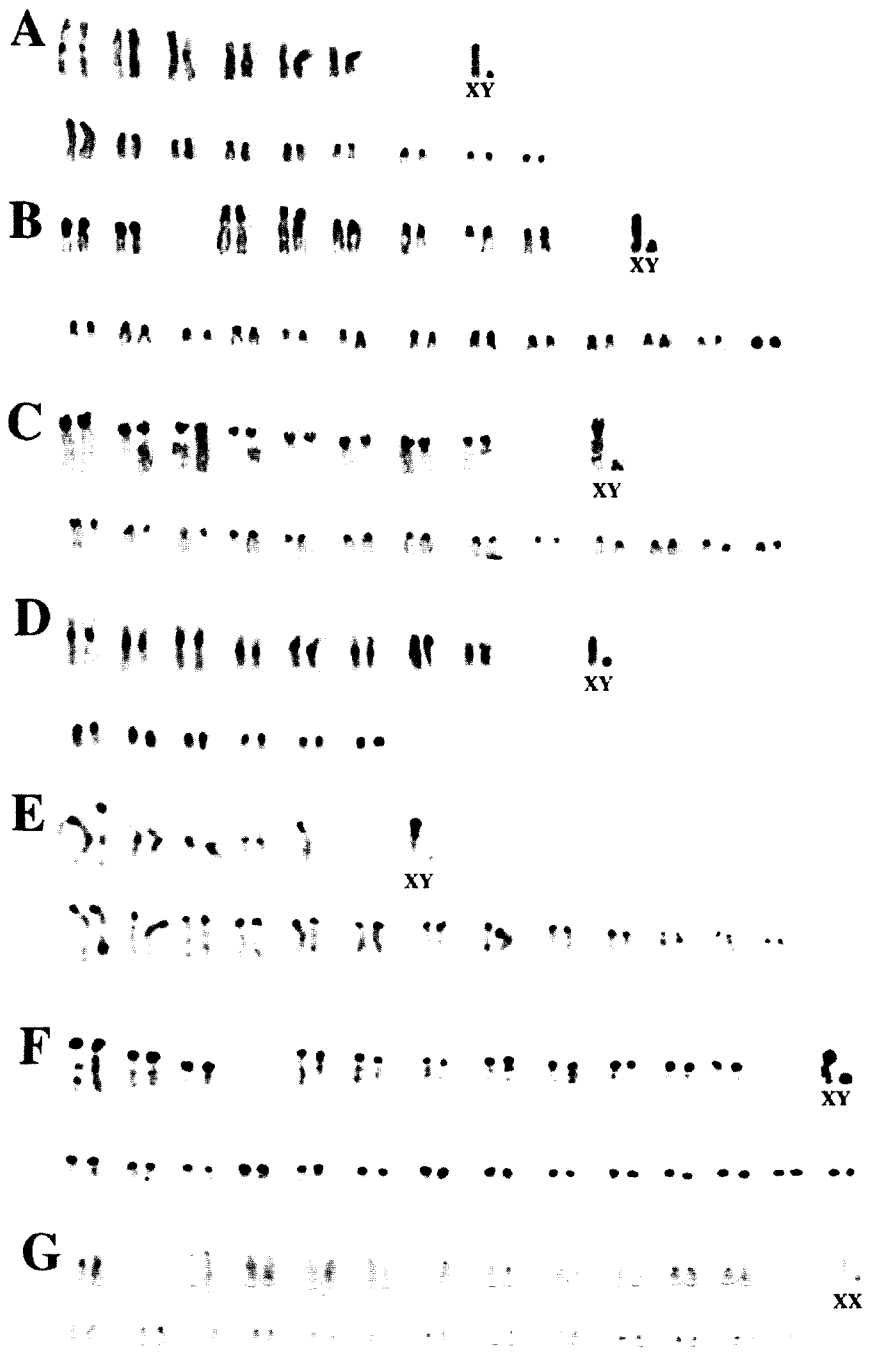

Fig. 2. C-banded complements of (**A**) *Calomyscus urartensis*, (**B**) *C. mystax*, (**C**) karyotype 3, (**D**) karyotype 4, (**E**) karyotype 5, (**F**) karyotype 6, and (**G**) karyotype 7.

of the autosomes possessed large C-heterochromatin blocks at the centromeres. The euchromatic parts of the acrocentrics were much smaller than the heterochromatic blocks. The X chromosome contained a heterochromatic short arm. The Y chromosome was completely heterochromatic. The karyotype was recorded in western and central Kopetdag and the Little Balkhan Mountains in Turkmenistan. Natural hybrids between individuals with karyotypes 3 and 4 were found in central Kopetdag.

Karyotype 5. A single male specimen from Bahtaran Province, Iran, presented a complement of 37 chromosomes and 44 autosomal arms (Figs. 2E and 3E), including three pairs of biarmed autosomes and 13 pairs of acrocentrics. G-banding indicated that the single large submetacentric element corresponded to two subtelocentric chromosomes homologous to each long and short arm, respectively. The heterochromatic short arm of one of the subtelocentric chromosomes was apparently missing in the fused submetacentric chromosome. All of

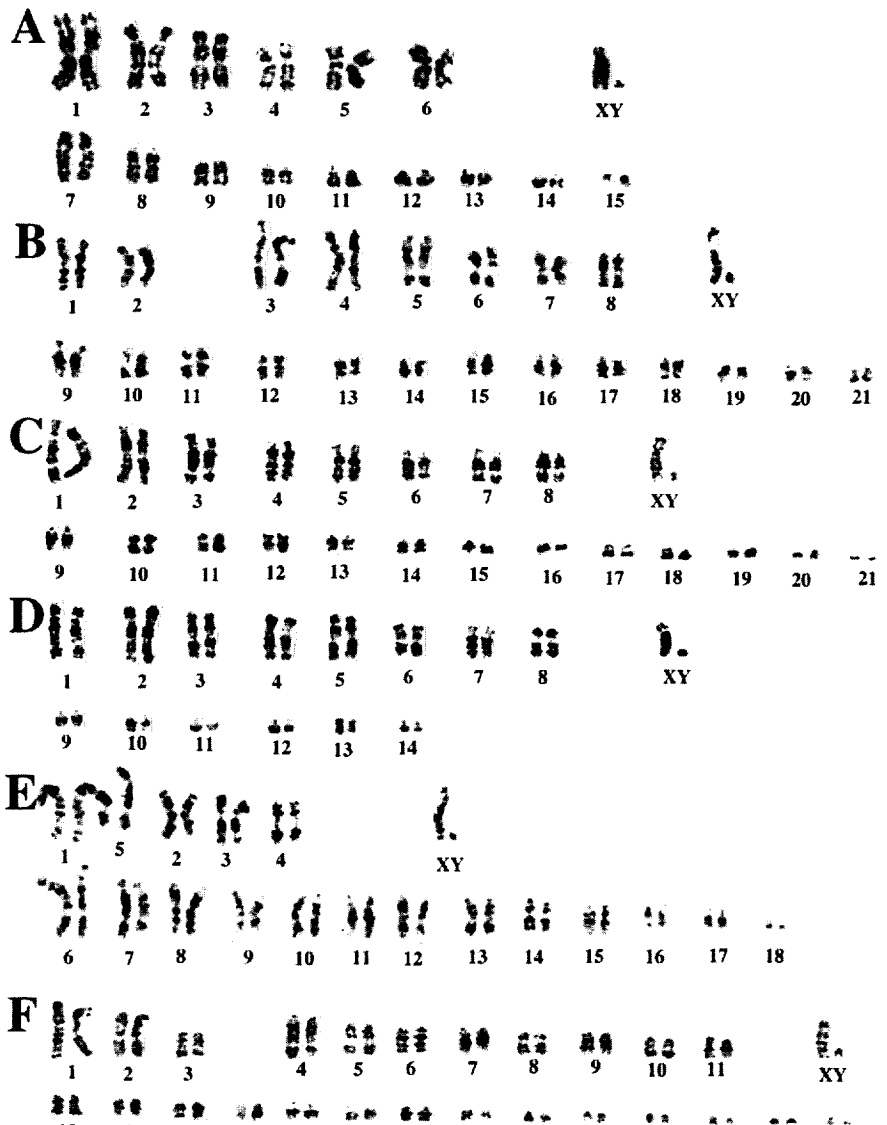

Fig. 3. G-banded complements of (**A**) *Calomyscus urartensis*, (**B**) *C. mystax*, (**C**) karyotype 3, (**D**) karyotype 4, (**E**) karyotype 5, (**F**) karyotype 6, and (**G**) karyotype 7.

the autosomes possessed small C-heterochromatin blocks at the centromeric regions and small heterochromatic arms. The X chromosome contained a heterochromatic short arm. The Y chromosome was a small element.

Karyotype 6. A single female specimen from Kerman Province, Iran, presented a complement of 52 chromosomes and 56 autosome arms (Figs. 2F and 3F). All of the autosomes were subtelocentric or acrocentric, and three had small heterochromatic arms; the others possessed large, dark C-bands at the centromeres. The X chromosome had a heterochromatic short arm. The Y chromosome was completely heterochromatic.

Karyotype 7. A diploid number of 50 chromosomes was found in the complement (Figs. 2G and 3G), including one biarmed pair and 23 pairs of acrocentric autosomes (FNa = 50). The presumptive X chromosome was acrocentric. The amount of C-heterochromatin was very low, and small pericentromeric bands were present in only a few autosomes. The karyotype was found in a single locality in Fars Province, Iran.

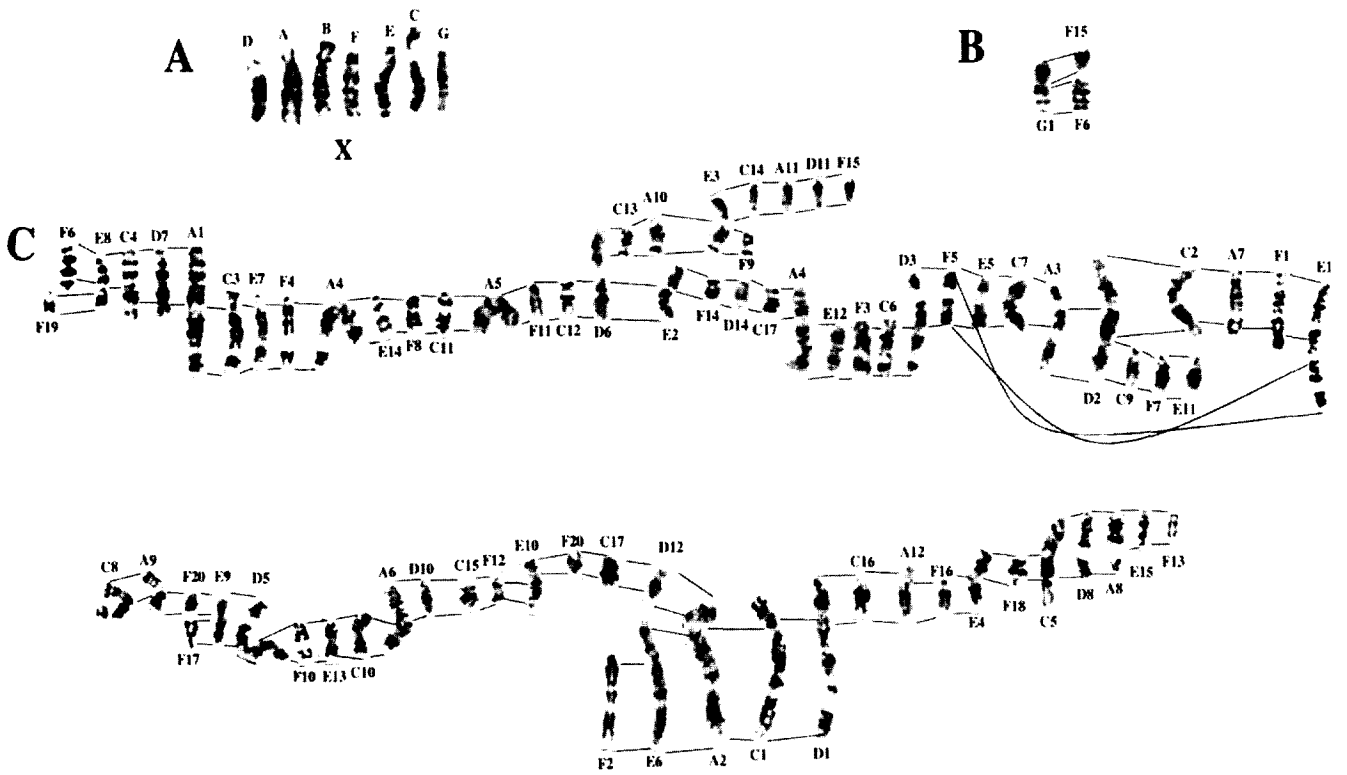

Fig. 4. Comparison of G-banded karyotypes of *Calomyscus* species. (**A**) X chromosomes of (A) *C. urartensis*, (B) *C. mystax*, (C) karyotype 3, (D) karyotype 4, (E) karyotype 5, (F) karyotype 6, and (G) karyotype 7. (**B**) Robertsonian rearrangement differentiating karyotypes 6 and 7. (**C**) Comparison of haploid complements of (A) *C. urartensis*, (C) karyotype 3, (D) karyotype 4, (E) karyotype 5, (F) karyotype 6, and (G) karyotype 7. Bars connecting the chromosomes delineate regions of homology.

Fig. 5. Primary structure of the 353-bp *Bsp*RI repeat of *Calomyscus*.

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GGATCCCCAG[10] AAACACACTC[20] CTTCTTACTA[30] GTTCTCACCT[40] CTGCAACATG[50]
TATTCAGAAG  GTGCTAAGCA   ACGTCATCTC   CAAACCGGCA  AACGCATATG
TTCATATTGC[110]AATACTTAAC[120] AGGACACTTT[130]TCTTCCAAGA[140]GCAAGACGCT[150]
ATCAAAGTAA  GCGAAACATC   ACATATCTCA  ACTATGTGAG  GGGGACTTGC
TAAGCGTCGC[210]TCTAACTTCA[220]GGAGCACTGC[230]CTTTTCCTAG[240]GCGTAGGTAA[250]
GCATTAGCTT  TGCTGCTTGT   GTGTAGTAAT  GGCTATTCTC   ATTGAAGCCA
TTTGCGCTCT[310] AATGGATCAA[320]GCAACACACT[330]ATGTAGCGAG[340]AGTGAGGAGCTG[353]
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Comparison of individual karyotypes

Comparative analysis of G-banded chromosomes revealed a high level of conservation in their banding patterns (Fig. 4A–C). The X chromosome showed an identical G-banding pattern in all species and populations studied; the variation in the size of the X chromosome reflected the presence or absence of heterochromatic arms (Fig. 4A). Karyotypes 6 and 7 differed by only a single centromeric fusion or fission (Fig. 4B) and in the character of the C-positive bands.

The complements recorded in *C. mystax* and in a male from Tehran (karyotype 1) were completely identical, and karyotype 3 was also quite similar to these complements. Chromosome divergence of these populations was accompanied by changes in the size and location of C-positive bands. The karyotypes with lower diploid numbers could be derived through centric and tandem fusions (Fig. 4C), however, the direction of chromosomal evolution cannot be assumed *a priori*.

Chromosome localization of Bsp*RI tandem repeat*

Electrophoretic patterns of digests were similar in all the specimens examined, and Southern analyses indicated that the 350-kb *Bsp*RI probe repeat recognized a tandem array in all specimens examined. We determined the nucleotide sequence of this repeat (EMBL Accession No. X59439, Fig. 5). This *Calomyscus*-specific repeat is not homologous to any known repeats isolated from rodents or other mammals.

Results of in situ hybridization of the *Calomyscus* karyotypes with the 350-kb *Bsp*RI repeat are shown at Fig. 6. This sequence was predominantly located at the centromeres of almost all of the chromosomes and in the heterochromatic arms. Therefore, the location of this repeat on the chromosomes coincides with their C-band pattern.

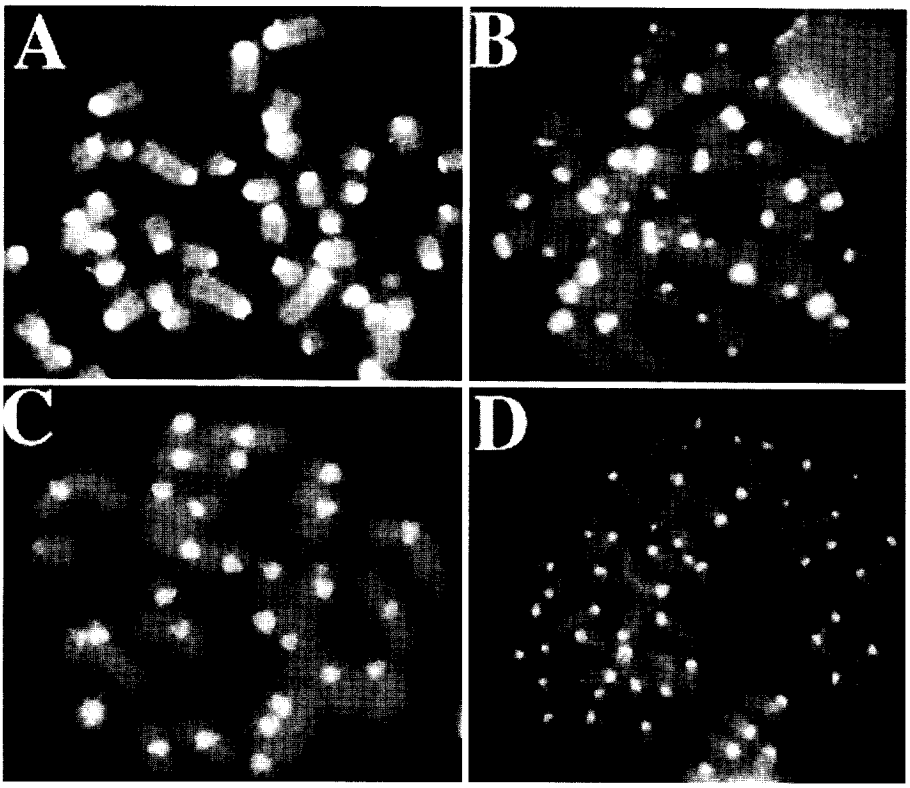

Fig. 6. Metaphase complements of (**A**) *Calomyscus mystax*, (**B**) karyotype 3, (**C**) karyotype 4, and (**D**) the hybrid between *C. urartensis* and karyotype 3, following FISH with the 353-bp *Bsp*RI repeat DNA probe.

Synaptonemal complexes and interspecific hybrids

In general, the appearance of meiotic prophase spermatocytes in the studied hybrids was similar to that described for the Chinese hamster (Moses, 1980). A specific feature of the morphology of the pachytene cells of *Calomyscus* was the appearance of the X-Y pair. The pairing region of the sex bivalent was typically like of the synaptonemal complex (SC) of an autosomal bivalent. The unpaired segments of the sex bivalent were much thinner and had a very low staining intensity. They were surrounded by a light cloud of electron-dense material.

Karyotype 2 differs from that of *C. mystax* in the presence of heterochromatic arms in eight autosomes; therefore, we expected to detect eight heteromorphic bivalents in the SC complement from hybrids between these forms. We found, however, that all the bivalents were completely paired, and additional unpaired arms were not observed. Moreover, neither asynapsis or delayed synapsis was detected. The autosomal bivalents of the hybrid between *C. mystax* and the karyotype 3 individual were identical to those of the parental species.

Karyotype 4 and that of *C. mystax* differ by seven Robertsonian translocations. The hybrid males demonstrated complete chromosome pairing in meiosis. In pachytene cells of these hybrids we found seven Robertsonian trivalents, each of them involving a metacentric and two homologous twin acrocentrics (Fig. 7). Usually, the synapsis between the metacentrics and the twin acrocentrics was complete.

Unfortunately, the most interesting hybrid, between the specimen with karyotype 4 and *C. urartensis* (a complex Robertsonian heterozygote with monobrachial homology), was

Fig. 7. Electron microphotograph of pachytene cell of a hybrid male between *C. mystax* and karyotype 4. Arrows indicate side arms of the Robertsonian trivalent.

Fig. 8. Chromosomes in diakinesis at metaphase I of a hybrid male between *C. urartensis* and karyotype 4.

not available for SC analysis. In air-dried meiotic chromosome preparations of this hybrid we found diakinesis MI cells containing four multivalents (Fig. 8). This confirms the existence of monobrachial homology between the two karyotypes which was indicated in the G-banding analysis.

Discussion

Comparative chromosome analyses of *Calomyscus* revealed a spectacular pattern of karyotypic differentiation between individuals and populations. The phylogenetic analysis of the observed pattern is complicated by the lack of additional data from other populations and by difficulties in finding an appropriate outgroup. Comparative cytogenetics should attempt to use the outgroup method to determine the primitive and derived state of chromosome rearrangements (Qumsiyeh and Baker, 1988). The choice of an appropriate outgroup, usually representing a closely related taxon, is fraught with difficulty in the case of *Calomyscus*. This genus represents an isolated lineage, relegated to a separate monotypical subfamily, the Calomyscinae, by Musser and Carleton (1993). Various Palaearctic species of hamsters possess karyotypes with usually low diploid numbers (Gamperl et al., 1978; Schmid et al., 1986; Haaf et al., 1987), which are considerably different from those found in *Calomyscus*. Based on external appearance, *Calomyscus* has been associated with New World sigmodontines (see Vorontsov and Potapova, 1979; Carleton and Musser, 1984). Conventionally stained and banded karyotypes of *Calomyscus* actually resemble those of the American deer mouse, *Peromyscus*, particularly in the presence and variation of the heterochromatic small arms (e.g., Robbins and Baker, 1981; Rogers et al., 1984; Greenbaum et al. 1994). However, we have not found any clear G-band homologies between the karyotypes of *Calomyscus* and *Peromyscus*. This observation supports the isolated phylogenetic position of the *Calomyscus* genus, but it complicates the use of a *Peromyscus* species as the outgroup.

The geographical pattern of distribution of individual recorded karyotypes is still incomplete, and it also does not enable a detailed reconstruction of the course of chromosome evolution. The range of *C. urartensis* is apparently restricted to the Transcaucasian area, whereas *C. mystax* occurs in the Great Balkhan Mountains. The systematic position of an individual with the same karyotype from Tehran is not clear. The karyotypic identity of these two allopatric populations can be explained either as persistence of the ancestral karyotype in different areas of the range or as a consequence of homoplasy. The Kopetdag Mountains in Turkmenistan and Iran are inhabited by populations with two distinct karyotypes, and natural hybrids were found in a contact area. The other karyotypes, recorded in Iran, were found in individual specimens originating from single localities, and the range of their distribution therefore cannot be estimated. Further investigations of individuals from additional populations are necessary to reveal the overall pattern of distribution. Nomenclatural problems related to the possible existence of undescribed species can be addressed only after examination of the type locality of *C. bailwardi* in southern Iran.

In a great number of groups, a high correlation between chromosomal differentiation and species diversity has been found (King, 1993). Does the extensive karyotypic variation recorded in *Calomyscus* indicate recent speciation events and/or is it just a manifestation of intraspecific polymorphism caused by chromosomal repatterning? Only the negatively heterotic chromosome changes provide the structural basis for reproductive isolation by effecting fertility (Patton and Sherwood, 1983; King, 1987). The chromosomal differences between the individuals and populations studied were caused by centric and tandem fusions or fissions and by additions and deletions of heterochromatin. There is little evidence to support the involvement of heterochromatin changes in speciation (John and Miklos, 1979). Tandem and centric fusions represent the rearrangements which could be negatively heterotic; if any of these rearrangements produce a balanced meiotic system, however, they will not be able to form a post-mating isolation mechanism. Our data do not indicate the presence of an effective reproduction barrier between individuals or populations of *Calomyscus* with distinct karyotypes. The record of natural hybrids between individuals with the karyotypes 3 and 4, as well as the existence of viable laboratory hybrids, suggest close relationships between individuals from chromosomally differentiated populations. This view is also supported by observations of the regular course of meiosis in the hybrids and by the finding of a highly specific *Bsp*RI tandem repeat.

Numerous models suggest that social structuring, vagility, and population size are all factors affecting the rate of chromosomal change (Wilson et al., 1975; Bush et al., 1977; Walsh, 1982). Our field experience indicates that populations of *Calomyscus* are distributed in a patchy mosaic pattern, and geographical isolation of individual populations is fairly common. This population structure obviously promotes the effect of random genetic drift and may be an important factor of rapid karyotype evolution. We can thus conclude that there is no

clear evidence suggesting the role of chromosomal change in speciation of the *Calomyscus* populations studied. Further data from contact areas between populations with distinct karyotypes are needed to elucidate the problem of evolutionary significance of structural karyotype repatterning in the *Calomyscus* genus.

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