Comparative Cytogenetics of Some Species of *Crocridura* (Insectivora) with $2n=40$

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Summary A GTG-banding was used for a detailed comparative analysis of two 40-chromosome *Crocridura* species, *Crocridura gueldenstaedtii* and *Crocridura dsinezumi*. A high level of chromosomal homology was detected. A complex 520-bp EcoRI tandem repeat was isolated from *C. dsinezumi*. Using fluorescence *in situ* hybridization (FISH), this repeat was localized to centromeric regions of chromosomes in both *Crocridura* species.

Key words *Crocridura dsinezumi*, *Crocridura gueldenstaedtii*, CDZ repeat, FISH, Comparative analysis, Chromosomal homology.

Today, there are more than 150 known species of the genus *Crocridura*. Among the 40 that have been karyotyped, only a few have been analyzed using C- and G-banding techniques. In the genus *Crocridura* the chromosome numbers have been found to range from 22 (*Crocridura pergrisea*) in Palearctic and Oriental species to 60 (*Crocridura bicolor*) in African species (Maddalena and Ruedi 1994). Most of the African species have a high diploid number (more than 40), whereas all Palearctic and Oriental species contain 40 chromosomes or less. Chromosomal comparison using differential staining of several *Crocridura* species assumed an ancestral karyotype of the genus having 36–40 chromosomes (Maddalena and Ruedi 1994). The evolution of the genus *Crocridura* may have proceeded in two opposite directions: the low chromosome Eurasian species may have evolved with a reduction in diploid number (Robertsonian and tandem fusion), while in the higher chromosomal African species, evolution may have occurred from an increase in both diploid and fundamental numbers due to fission (Graphodatsky *et al.* 1988, Maddalena and Ruedi 1994, Harada *et al.* 1985).

Many of the Eurasian species of *Crocridura* have 40 chromosomes. Graphodatsky *et al.* (1988) regarded such a number as primitive among the Palearctic-Oriental (Eurasian) species. To better understand the ancestral karyotype, a comparison of 40-chromosome species would be useful. For three Palearctic 40-chromosome species, *Crocridura suaveolens*, *Crocridura sibirica* and *Crocridura gueldenstaedtii*, G- and C-banding revealed complete homology of the autosome sets and the X chromosomes (Graphodatsky *et al.* 1988). Differing only slightly phenotypically while appearing to have the same karyotype, these three species are often classified as a single group named *Suaveolens* (Graphodatsky *et al.* 1988, Zaitsev 1989). The G- and C-band karyotypes of the Oriental 40-chromosome *Crocridura dsinezumi* has been previously described by Harada *et al.* (1985).

Here we present a comparison of GTG- and C-band karyotypes of the Oriental *C. dsinezumi* and Palearctic *C. gueldenstaedtii* (the *Suaveolens* group). A 520-bp EcoRI complex tandem repeat

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was isolated from *C. dsinezumi*. Using fluorescence *in situ* hybridization (FISH) it was localized to the chromosomes of both *Crocidura* species.

Material and methods

Banding analysis

Metaphase chromosomes were prepared from bone marrow cells that were suspended in 199 tissue culture medium supplemented with 10% calf serum and incubated for 2 h at 37°C with colcemid (0.02 µg/ml) for 30 min. The cells were swollen in 0.04 M KCl for 15 min, fixed in ice-cold methanol-acetic acid fixative, spread on moist cold slides and air-dried. G-band staining was performed according to the method of Seabright (1971), as modified by Graphodatsky and Radjabli (1988). Slides were treated with a 0.025% trypsin solution in isotonic saline for 1–2 min at room temperature, rinsed in 2×SSC and stained for 5 min in 4% Giemsa solution. C-band staining followed the modified version of Sumner (1972). Slides were incubated in 5% Ba(OH)₂ for 5 min, then in 2×SSC for 1 h at 62.5°C before staining in 4% Giemsa for 30 min.

DNA isolation and sequencing

DNA was isolated from liver and a restriction enzyme digestion performed using standard procedures (Sambrook *et al.* 1989). Highly repeated sequences were isolated by digestion of the total DNA of *C. dsinezumi* with the restriction enzyme EcoRI resulting in a 520-bp fragment. The DNA fragment was isolated by electroelution, cloned in the EcoRI site of a pUC19 vector and transformed into an *E. coli* strain DHSa. Transformants were identified and manually sequenced using appropriate primers by the dideoxynucleotide chain-terminal method (Sanger *et al.* 1977) and the Sequenase Version 2.0 system (USB).

The DNA fragment was signed as CDZ and its genomic sequence was deposited in the EMBL Database under Accession Number Y14806.

Southern blot hybridization

C. dsinezumi was tested for repeat sequences by Southern blot analysis. 5 µg of DNA was digested with EcoRI, fractionated on 1.5% agarose gel, and transferred to a Hybond N membrane (Amersham). Plasmid probes were labeled with ³²P by random priming. Hybridizations were performed at 55°C in 6×SSC, 0.5% SDS, 100 mg/ml heparin and 100 mg/ml salmon sperm DNA, and washed at 65°C in 0.1×SSC and 0.1% SDS.

Repeat DNA bands corresponding to the cloned fragment and its multimers were taken as DNA ladders.

FISH

Fluorescence *in situ* hybridization (FISH) was performed according to Pinkel *et al.* (1986). Hybridization of a biotin-labeled probe of CDZ repeat to *Crocidura* chromosomes was detected with fluorescein-conjugated avidin. Chromosomes were counterstained with propidium iodide. Photographs were taken on Kodak 400 ASA film.

Results and discussion

Comparative analysis of G- and C-banded chromosomes

G- and C-banded chromosomes of *C. dsinezumi* (Figs. 1b, 2b) were arranged according to Harada *et al.* (1985), and the chromosomes of *C. gueldenstaedtii* (Figs. 1a, 2a) according to Graphodatsky *et al.* (1988). For comparative analysis (Fig. 3), we used GTG-banded chromosomes of *C. dsinezumi* and *C. gueldenstaedtii* at the same level of resolution.

Fig. 1. G-banded karyotypes of *C. gueldenstaedtii* (a) and *C. dsinezumi* (b).

The centromeric C-heterochromatin blocks of different sizes were located on most of the autosomes and on the X chromosome of *C. gueldenstaedtii* (Fig. 2a). All chromosomes of *C. dsinezumi* (Fig. 2b), except the submetacentrics 4, 5 and 7, displayed small blocks of centromeric C-heterochromatin. The X chromosome of *C. dsinezumi* had an additional telomeric C-band relative to the *C. gueldenstaedtii* X chromosome. The Y chromosomes of both species were uniformly dark.

Comparison of G-band karyotypes revealed a high level of homology between the chromosomes of *C. dsinezumi* and *C. gueldenstaedtii* (Fig. 3). Nine of the 19 pairs of autosomes of *C. dsinezumi* were identical to nine pairs of chromosomes of *C. gueldenstaedtii*. Submetacentrics 5, 2 and 3 of *C. dsinezumi* were homologous to submetacentrics 1, 3 and 4 of *C. gueldenstaedtii*, respectively. Acrocentrics 9, 11, 10, 13, 15, and 17 of *C. dsinezumi* were identical to acrocentrics 6, 10, 12, 16, 14, and 17 of *C. gueldenstaedtii*, respectively. The q-arm of subtelocentric 2 of *C. gueldenstaedtii* was homologous to *C. dsinezumi* acrocentric 12, whereas the p-arm of subtelocentric 2 was

Fig. 2. C-banded karyotypes of *C. gueldenstaedtii* (a) and *C. dsinezumi* (b). Since it is impossible to distinguish the C-banded acrocentrics, only the bigger and smallest acrocentrics were numbered.

completely heterochromatic.

The following arm homology was detected. The q-arms of the *C. dsinezumi* chromosomes 4 and 6 were homologous to acrocentrics 5 and 9 of *C. gueldenstaedtii*, respectively. The *C. dsinezumi* chromosomes 16 and 19 appeared to be similar to chromosome 11 of *C. gueldenstaedtii*.

We found monobrachial homology between the *C. dsinezumi* chromosomes 1 and 18 and chromosomes 7 and 15 of *C. gueldenstaedtii*. The q-arm of chromosome 1 of *C. dsinezumi* was homologous to acrocentric 15 of *C. gueldenstaedtii*, whereas the p-arm of chromosome 1 and acrocentric 18 of *C. dsinezumi* were similar to chromosome 7 of *C. gueldenstaedtii*.

The remaining three pairs of chromosomes displayed extensive regions of homology. *C. dsinezumi* chromosome 7 had a similar banding pattern to the major part of acrocentric 13 of *C. gueldenstaedtii*. The banding pattern of the proximal part of the chromosome 8 of *C. dsinezumi* corresponded to that of the distal part of chromosome 18 of *C. gueldenstaedtii*, whereas the rest of *C. dsinezumi* chromosome 8 was homologous to *C. gueldenstaedtii* chromosome 8. The distal two thirds of acrocentric 14 of *C. dsinezumi* corresponded to acrocentric 19 of *C. gueldenstaedtii* except for an inverted position.

The small proximal parts of *C. dsinezumi* chromosomes 4, 6 and 14 were probably homo-

Fig. 3. Comparison of haploid complements of *C. dsinezumi* (large figures) and *C. gueldenstaedtii* (small figures). Outside bars indicate regions of homology

gous to the proximal parts of *C. gueldenstaedtii* chromosomes 13 and 18, but they were too small for this to be determined in details.

The GTG-banding pattern of the X chromosomes differed substantially. Several rearrangements such as pericentric inversion and centromeric shift have possibly taken place in the evolution of the X chromosome. The Y chromosomes were both acrocentrics, with the Y chromosome of *C. gueldenstaedtii* being twice as long as the Y chromosome of *C. dsinezumi*. Such a size difference could be due to the variation in the amount of C-heterochromatin (Harada *et al.* 1985, Graphodatsky *et al.* 1988, this study).

Thus, the GTG-banding revealed a high level of homology between Oriental and Palearctic 40-chromosome species. The regions of homology and homologous chromosomes that have remained conserved during chromosomal evolution could be elements of Eurasian *Crocidura* ancestral karyotype. If the ancestral karyotype consisted of more than 40 chromosomes, the chromosome evolution may have involved only tandem fusion and whole arm reciprocal translocations. If the ancestral karyotype consisted of 40 chromosomes, more rearrangements would have been needed, including not only fusion and whole arm reciprocal translocations, but also fission. To clarify this, a greater number of species must be compared.

Most of the conserved chromosomes, or part of the chromosomes detected, are present also in the hypothetical ancestral karyotype proposed by Maddalena and Ruedi for the whole genus *Crocidura* (1994).

Chromosomal localization of the CDZ repeat

Southern analyses indicated that the EcoRI CDZ repeat probe of 520-bp recognized a tandem

GAATTCACCT	GTGGTAAAA	GATTCTTCTC	CTTCTTGAA	ACTGCTTT GT	CGAAGCTTGT
ACAACTCGGC	AGTGATCTCT	GTGTTCTTCC	TACAGAGTTC	TGAGCACATT	TGCGTGGCCG
ACGAACATTA	CACAATTGAG	AACTTAACAT	GCGGTTTCA	TCAAGATTCT	TCAATTTTCA
GTTTCTCAIT	TCACGTITCT	TTTCCCTTCT	GTTCTAATGC	AATGTGTATG	TTTCTATATA
TTCTGCGCAT	TCAGTCATCT	CCTTCTTTGA	TTTTGATGTG	TTACTGATGT	TCTTCTTCT
TTGCTCATAA	CTTTACACCC	CGAAGTGTTT	TCTGTGTTAT	TCTAATGCTG	TCTTAATACT
TTTGCATCTC	CCTTTAAAA	CTACACATTT	GACAGCAGAA	AATGTTGTGT	TCTTCATAAT
ACTTGATTAT	TCACGTTTGA	TTTCATTGAA	TGCAAGTTTC	TGTTGTAATT	TGATGTGAAT
ATTGCTTGTT	TTTACTGTAT	TCGTTTATCT	CGGTTTIT		

Fig. 4. The primary structure of the CDZ repeat, isolated from *C. dsinezumi*.

Fig. 5. Metaphase chromosomes following fluorescence *in situ* hybridization with the CDZ repeat DNA probe: *C. dsinezumi* (A), *C. gueldenstaedtii* (B).

array in *C. dsinezumi* and *C. gueldenstaedtii*. It was absent in the genomes of *Sorex araneus* and *Suncus murinus* (data not shown). The repeated nucleotide sequence is shown in Fig. 4. No homology was found between CDZ crocidura-specific repeats and any known repeated sequences isolated from *Insectivores* or any other mammals. The CDZ repeat appears to be genus specific, or even subgenus specific, regarding *Suncus* as subgenus of genus *Crocidura*.

Results of the *in situ* hybridization of the CDZ repeat to *Crocidura* Chromosomes (Fig. 5) demonstrate that *C. dsinezumi* and *C. gueldenstaedtii* share the same centromeric repeat. Most of the chromosomes of *C. dsinezumi* (Fig. 5A) displayed clear signals in their centromeric regions. In *C. gueldenstaedtii* the CDZ repeat was also located on the centromeric regions of chromosomes. The X and Y chromosomes of both species had signals only in the centromeric regions.

Both cytogenetic and molecular data favor *C. gueldenstaedtii* and *C. dsinezumi* having monophyletic origin and a recent common ancestry.

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