# A Comparative Analysis of the Karyotypes of *Cricetus cricetus* and *Cricetulus griseus*

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Abstract. This study presents a comparison of the mitotic chromosomes of the two species of hamsters Cricetus cricetus (European hamster) and Cricetulus griseus (Chinese hamster), which have the same chromosome number of 2n=22. — G-banding procedure reveals striking similarities in both karyotypes and gives the possibility to analyse structural changes so that two examples for Robertsonian rearrangement can be observed. — A remarkable kind of difference between the two karyotypes becomes obvious after C-banding procedure. While Cricetus shows a large amount of predominantly centromeric heterochromatin, in Cricetulus griseus C-bands are less conspicuous, and a few chromosomes do not exhibit any centromeric heterochromatin at all.

## Introduction

Because of several preceding investigations (e.g. Matthey, 1960, 1961; Hsu and Arrighi, 1966, 1968; Nadler, 1969; Turleau and de Grouchy, 1973; Stock and Hsu, 1973; Voiculescu, 1974), it is well known that phylogenetic and taxonomic relationships between different species can be made sure by karyological analysis. The recently developed staining techniques have provided the investigators with the means for a detailed comparison of different karyotypes. Now it is possible to analyse the chromosomes and even parts of chromosomes which are involved in changes.

Only a few mechanisms are described to be mainly responsible for chromosomal rearrangements: Robertsonian fusion, centric fission, pericentric inversion, duplication and deletion. In addition, C-staining technique has revealed another mechanism: the increase or loss of heterochromatic short arms that has been observed in the genera *Mesocricetus* (Voiculescu, 1974) and *Peromyscus* (Duffey, 1972; Pathak *et al.*, 1973).

In our own investigations, we have compared the chromosomes of the two hamster species *Cricetus cricetus* and *Cricetulus griseus*. Both karyotypes have already been described, that of *Cricetus cricetus* by us (Vistorin *et al.*, 1976), and that of *Cricetulus griseus* by several authors, among others by Hsu and Arrighi (1971), Kakati and Sinha (1972), Kato and Yosida (1972). The two hamster species correspond in their chromosome number of 2n=22, but differ in number and relative length of chromosomal arms. We have tried to analyse the similarities in G- and C-banding patterns and the possible changes that have led to the differences in the karyotypes.

### **Material and Methods**

Chromosome preparations were obtained from fibroblast cultures as described earlier (Vistorin *et al.*, 1976). More than 100 mitoses of each species were analysed after G- and C-banding procedure (Sumner *et al.*, 1971; Sumner, 1972).

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The European hamsters (6 males and 4 females) were captured in Eastern Austria. The Chinese hamsters were obtained from two institutes, some individuals from Institut für Biologische und Medizinische Forschung AG, Füllinsdorf, Switzerland, others from Institut für Zoologie I, Vienna. These hamsters were then bred in our laboratory. We have examined 6 male and 5 female Chinese hamsters. — The amount of heterochromatin in the karyotypes after C-banding procedure was determined by use of the Leitz-"Classimat".

## Results

Comparison of the karyotypes of *Cricetus cricetus* and *Cricetulus griseus* reveals striking similarities as far as G-banding pattern is concerned, but evident differences with regard to C-banding pattern. These differences affect above all the amount of heterochromatin, which is large in the complement of the European hamster (approximately 34%) and relatively small in that of the Chinese hamster (approximately 25%). A remarkable difference can be found concerning centromeric heterochromatin. In the karyotype of *Cricetus cricetus*, each chromosome shows a centromeric block after C-banding procedure (Fig. 1), whereas in the complement of *Cricetulus griseus*, there are three autosomes (nos. 1–3) which do not have any distinct centromeric heterochromatin (Fig. 2). This discrepancy in the distribution of heterochromatin must be considered, when it is tried to find out conformities in the two karyotypes.

In the following, we describe the presumed homologies between the two karyotypes, chromosome by chromosome, as they are arranged in Fig. 3.

Chromosome no. 1 of *Cricetus cricetus* shows a banding pattern very similar to chromosome no. 2 of *Cricetulus griseus*.

Chromosome no. 2 of *Cricetus cricetus* corresponds to no. 3 of *Cricetulus griseus*. The short arm of the European hamster chromosome shows good coincidence with the long arm of the Chinese hamster chromosome, whereas the differences in the remaining arms may be explained by the additional heterochromatin on the long arm of chromosome no. 2 of *Cricetus cricetus*.

Good homology exists between the banding patterns of the chromosomes nos. 4 of *Cricetus cricetus* and *Cricetulus griseus*. The difference in length is supposed to be due to the greater amount of heterochromatin in the European hamster chromosome.

The banding patterns of the submetacentric chromosomes nos. 5 and 6 of *Cricetus cricetus* are similar to that of chromosome no. 1 of *Cricetulus griseus*. We suppose that it is not only the Robertsonian process which was involved in the rearrangement, but that, in addition, inversions or deletions must have taken place. It is not yet possible for us to decide exactly, which kinds of change have taken part in the transformation.

Chromosome no. 3 of *Cricetus cricetus* has no homologous metacentric chromosome in the karyotype of *Cricetulus griseus*, but the two acrocentric chromosomes nos. 6 and 7 of the Chinese hamster may be matched each with one arm of the metacentric chromosome of the European hamster, no. 6 with the longer arm, no. 7 with the shorter one. It can be supposed that we here see a typical example for the Robertsonian fusion. This metacentric chromosome of the European hamster seems to be the same as it has been described for *Cricetulus barabensis* (Matthey, 1960; Radjabli and Kriukova, 1973), where the same



Fig. 1. Karyotype of the European hamster (Cricetus cricetus) after C-banding procedure (bar=10  $\mu m)$ 



Fig. 2. Karyotype of the Chinese hamster (Cricetulus griseus) after C-banding procedure (bar=10  $\mu$ m)



Fig. 3. Representation of G-banded chromosomes of Cricetus cricetus and Cricetulus griseus, arranged according to their supposed homology. E chromosomes of Cricetus cricetus, C chromosomes of Cricetulus griseus (bar=10 µm)

chromosomes nos. 6 and 7 of the Chinese hamster karyotype are suspected to have fused.

Chromosome no. 7 of *Cricetus cricetus* shows good homology with chromosome no. 5 of *Cricetulus griseus*.

Similarities can also be found between the small chromosomes nos. 8–10 of both hamsters. The differences that are to be seen in the nos. 8 and 9 are supposed to be due to the higher amount of centromeric heterochromatin in the European hamster.

The two sex chromosomes are difficult to compare, as their banding patterns are not well differentiated. The X-chromosomes of both hamsters show the two characteristic G-bands that have previously been described for several different hamster species. These two bands are localized on the euchromatic arms, that means that they are found on the long arm of the European hamster X-chromosome and on the short arm of the Chinese hamster chromosome. C-banding procedure reveals rather great differences between the sex chromosomes of both species. While X- and Y-chromosome of *Cricetus cricetus* have an intensively stained centromeric region and show different kinds of staining intensity (Fig. 1), this cannot be discerned in the sex chromosomes of *Cricetulus griseus*. The Xchromosome of *Cricetulus griseus* consists of an euchromatic short arm and an intensively stained heterochromatic long arm, and reveals a remarkable structure: two prominent, darkly stained spots in the middle of the long arm (Fig. 2). The Y-chromosome of *Cricetulus griseus* is darkly stained throughout its length.

## Discussion

The comparison of the karyotypes of Cricetus cricetus and Cricetulus griseus gives a further confirmation to the theory that Robertsonian fusion or centric fission is a very common mechanism involved in chromosomal rearrangements. In the case described above, we found two examples for this Robertsonian process, which is--strictly speaking-a centromere to centromere translocation. As in general, here as well it is difficult to decide, whether the process in question was a fusion or fission. Concerning the first example which affects the chromosomes nos. 5 and 6 of the European hamster and chromosome no. 1 of the Chinese hamster, the interpretation has been complicated by the fact that the Robertsonian process is obviously accompanied by inversions and (or) deletions. In the second case, the European hamster complement shows the metacentric chromosome (no. 3), whereas that of the Chinese hamster has two corresponding acrocentric chromosomes (nos. 6 and 7). Here a mere Robertsonian process is supposed to be present. As it has been mentioned above, the same metacentric chromosome can be found in the karyotype of *Cricetulus barabensis* and has been interpreted by Matthey (1960) and Radjabli and Kriukova (1973) to be the result of a fusion, though the inverse process cannot be excluded. It would be of interest, whether these metacentric chromosomes of Cricetus cricetus and Cricetulus barabensis, which show very similar G-banding patterns, correspond in C-banding pattern as well.

Apart from the Robertsonian process, there is another kind of transformation that has led to remarkable differences in the two karyotypes: the alteration of the amount and distribution of heterochromatin. The changes observed here are in a certain contrast to several other cases described previously, where it is heterochromatin as well which plays an important role in evolution. For example in the genera *Peromyscus* (Duffey, 1972; Pathak *et al.*, 1973) and *Mesocricetus* (Voiculescu, 1974), additional amounts of heterochromatin are mainly localized in supplementary heterochromatic short arms of several chromosomes, which led to differences in the fundamental number (F.N.) of arms in related species. Our investigations have revealed that the increased amount of heterochromatin may as well be localized mainly in the centromeric region of the chromosomes so that the fundamental number must not be changed by the enlargement of the heterochromatic part of the genome.

The difference in the F.N. between the two hamster species (F.N.=42 for the European hamster, F.N.=40 for the Chinese hamster) can be explained by the supposed inversions that have taken place in the course of the transformation in which the chromosomes nos. 5 and 6 of *Cricetus cricetus* and chromosome no. 1 of *Cricetulus griseus* were involved. — Besides the additional amount of heterochromatin located in the centromeric region, there have been observed supplementary interstitial heterochromatic bands in the chromosomes of the European hamster. Such variable amounts of interstitial heterochromatin in related species have as well been found for example in bats (Stock, 1975) and *Cetacea* (Arnason, 1974).

As it has previously been mentioned (Vistorin *et al.*, 1976), we suppose that the different staining intensities after C-banding procedure are an indication for different categories of heterochromatin, present in the chromosomes of the European hamster. In the karyotype of the Chinese hamster, different staining intensities after C-banding procedure cannot be discerned with sufficient certainty, so that it may be assumed that *Cricetulus griseus* does not have the same categories of heterochromatin as *Cricetus cricetus*. On the other hand Arrighi *et al.* (1974) showed that some of the heterochromatic segments of *Cricetulus griseus* appear to contain highly repetitive DNA, while others consist of less highly repetitive fractions. But a definite statement cannot be made before investigations on the composition of repetitive DNA sequences in both species have been carried out.

In the course of our investigations, we have found another difference between the two hamster species which concerns polymorphism. While polymorphism was not uncommon in *Cricetus cricetus*, we did not detect any case of polymorphism in *Cricetulus griseus*, though the examined individuals were obtained from different lines, and showed slight morphological differences, *e.g.* in coat colour. In *Cricetus cricetus*, polymorphism always affected the amount of heterochromatin, in sex chromosomes as well as in autosomes. As heterochromatin probably does not contain structural genes, it can be supposed that variations in the amount of heterochromatin do not affect the present viability, but—in the long run—may favour species formation.

With regard to the origin of these two hamster species, we could not find precise statements in the literature. According to Schaub (1930), we may assume that they evolved approximately during the same period of time, so that it is impossible to decide, which species is the ancestral one. Further investigations of other hamster species may perhaps throw more light upon the trend of species formation.

Acknowledgement. These investigations were supported by Fonds zur Förderung der wissenschaftlichen Forschung, Wien.

#### References

Árnason, Ú.: Comparative chromosome studies in Cetacea. Hereditas (Lund) 77, 1-36 (1974)

- Arrighi, F. E., Hsu, T. C., Pathak, S., Sawada, H.: The sex chromosomes of the Chinese hamster: constitutive heterochromatin deficient in repetitive DNA sequences. Cytogenet. Cell Genet. 13, 268-274 (1974)
- Duffey, P. A.: Chromosome variation in Peromyscus: a new mechanism. Science 176, 1333-1334 (1972)
- Hsu, T. C., Arrighi, F. E.: Chromosomal evolution in the genus Peromyscus (Cricetidae, Rodentia). Cytogenetics 5, 355–359 (1966)
- Hsu, T. C., Arrighi, F. E.: Chromosomes of Peromyscus (Rodentia, Cricetidae). I. Evolutionary trends in 20 species. Cytogenetics 7, 417–446 (1968)
- Hsu, T. C., Arrighi, F. E.: Distribution of constitutive heterochromatin in mammalian chromosomes. Chromosoma (Berl.) 34, 243-253 (1971)
- Kakati, S., Sinha, A. K.: Banding patterns of Chinese hamster chromosomes. Genetics 72, 357-362 (1972)
- Kato, H., Yosida, T. H.: Banding patterns of Chinese hamster chromosomes revealed by new techniques. Chromosoma (Berl.) 36, 272–280 (1972)
- Matthey, R.: Chromosomes, hétérochromosomes et cytologie comparée des Cricetinae paléarctiques (Rodentia). Caryologia (Firenze) 13, 199–223 (1960)
- Matthey, R.: Cytologie comparée des Cricetinae paléarctiques et américains. Les chromosomes de Calomyscus bailwardi Thomas et la position du genre Calomyscus dans la systématique des Cricetinae. Rev. suisse Zool. 68, 41-61 (1961)
- Nadler, C. F.: Chromosomal evolution in rodents. In: Comparative mammalian cytogenetics (K. Benirschke, ed.), p. 277–309. Berlin-Heidelberg-New York: Springer 1968
- Pathak, S., Hsu, T. C., Arrighi, F. E.: Chromosomes of Peromyscus (Rodentia, Cricetidae). IV. The role of heterochromatin in karyotypic evolution. Cytogenet. Cell Genet. 12, 315–326 (1973)
- Radjabli, S. I., Kriukova, E. P.: A comparison of banding patterns in two hamster species: Cricetulus barabensis and Cricetulus griseus. Tsitologiya 15, 1527–1531 (1973)
- Schaub, S.: Quartäre und jungtertiäre Hamster. Abh. schweiz. paläont. Ges. Zürich 49, 1-49 (1930)
- Stock, A. D.: Chromosome banding pattern homology and its phylogenetic implications in the bat genera Carollia and Choeroniscus. Cytogenet. Cell Genet. 14, 34–41 (1975)
- Stock, A. D., Hsu, T. C.: Evolutionary conservativism in arrangement of genetic material. A comparative analysis of chromosome banding between the Rhesus macaque (2n=42, 84 arms) and the African green monkey (2n=60, 120 arms). Chromosoma (Berl.) 43, 211-224 (1973)
- Sumner, A. T.: A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. 75, 304–306 (1972)
- Sumner, A. T., Evans, H. J., Buckland, R.: New technique for distinguishing between human chromosomes. Nature (Lond.) New Biol. 232, 31-32 (1971)
- Turleau, C., de Grouchy, J.: New observations on the human and Chimpanzee karyotypes. Identification of breakage points of pericentric inversions. Humangenetik 20, 151–157 (1973)
- Vistorin, G., Gamperl, R., Rosenkranz, W.: Analysis of mitotic and meiotic chromosomes of the European hamster, Cricetus cricetus (L.). Z. Säugetierkde (in press, 1976)
- Voiculescu, I.: A comparative study of the chromosome banding patterns of Mesocricetus newtoni and Mesocricetus auratus. Z. Säugetierkde 39, 211–219 (1974)

Received November 28, 1975 / Accepted December 18, 1975 by H. Bauer Ready for press December 19, 1975