

SHORT PAPERS

Constitutive heterochromatin and karyotype variation in Indian pygmy mouse, *Mus dunnii*

BY T. SHARMA AND G. S. GARG

*Cytogenetics Laboratory, Department of Zoology,
Banaras Hindu University, Varanasi-221005, India.*

(Received 5 January 1975)

SUMMARY

The Indian pygmy mouse, *Mus dunnii*, exhibits great variation in the number of chromosome arms while its diploid number of chromosomes remains constant. The variation seems to be due to addition or deletion of C-band positive constitutive heterochromatin in the short arms of autosomes.

1. INTRODUCTION

A karyological and taxonomic study of pygmy mice collected from a rice field in the neighbourhood of Madras in South India by Matthey & Petter (1968) had revealed that there were two morphologically very similar species, *M. dunnii* and *M. booduga*, which occupied the same habitat but possessed divergent karyotypes. Although both species were reported to have $2n = 40$, all the chromosomes of *M. booduga* were acrocentric whereas *M. dunnii* had a large metacentric X accompanied by a large acrocentric Y and a variable number of submetacentric autosomes in the complement. In Varanasi in North India we also encountered two distinct karyotypes in morphologically hardly distinguishable pygmy mice collected from the same rice fields. These mice were identified as *M. booduga* and *M. dunnii*. The karyotype of *M. booduga* from Varanasi observed by us resembled exactly the karyotype of the same species from Madras studied by Matthey and Petter but, on the other hand, the karyotypes of *M. dunnii* from these two places differed considerably. The difference in the karyotypes seemed to be due to variation in the quantity of C-band positive constitutive heterochromatin of the autosomal short arms.

2. MATERIALS AND METHODS

A total of 29 mice belonging to *Mus dunnii* (18) and *M. booduga* (11) were collected by digging burrows in rice fields of Kanchanpur and Ramnagar villages near the Banaras Hindu University campus at Varanasi. The chromosome preparations were made from bone-marrow of colcemid injected individuals by the usual ignition method after hypotonic pretreatment and fixation. About 50 metaphase spreads were counted from each individual. The techniques of Arrighi & Hsu (1971) and Sumner (1972) were used for C-band staining. Taxonomic identification of the mice was kindly done by Dr Joe T. Marshall. The skins and skulls are kept in the laboratory after identification.

3. RESULTS

In both the species, *M. dunni* and *M. booduga*, the diploid number of chromosomes was 40. All the 40 chromosomes, including the X and Y, of *M. booduga* were acrocentric without a conspicuous second arm as in the specimens of the same species from Madras studied by Matthey & Petter.

The Karyotype of *M. dunni* from Varanasi was distinct from that of *M. dunni* from Madras by the conspicuous absence of the submetacentric type of autosome from the complement (Plate 1, fig. 1). Nevertheless, all the autosomes had easily discernible second arms, some of which were quite prominent. A large metacentric X, the largest in the complement, and a large acrocentric Y were, however, present in the Varanasi karyotype as in the Madras karyotype investigated by Matthey and Petter.

The C-band staining of *M. dunni* chromosomes showed that the minute but distinct short arms of all the autosomes were darkly stained (Plate 1, fig. 2). A few autosomal pairs also had intercalary C-bands. The sex chromosomes exhibited very characteristic patterns. The entire Y, except at its centromeric region, was deeply stained, and the complete short arm and the telomeric region of the long arm of the metacentric X were deeply stained.

4. DISCUSSION

The large-sized sex chromosomes invariably present in all the individuals of *M. dunni* studied from the Madras and Varanasi populations strongly suggest that the karyotype of *M. dunni* is well diversified from that of *M. booduga*, which has normal-sized acrocentric sex chromosomes. However, the variation from 7 to 10 in the number of submetacentric autosomes observed by Matthey and Petter in a small sample of *M. dunni* collected from Madras and lack of such submetacentric autosomes in the individuals studied from Varanasi by us clearly indicate that in *M. dunni* further diversification of the karyotype is in progress.

A large number of species of American deer mice belonging to the genus *Peromyscus* are well known to exhibit inter- and intra-species variation in the number of chromosome arms although they consistently have the same diploid number (Hsu & Arrighi, 1966, 1968; Lee, Schmidly & Huheey, 1972). The variation in the number of chromosome arms of *Peromyscus* has been demonstrated to be due to addition or deletion of constitutive heterochromatin (Duffey, 1972; Bradshaw & Hsu, 1972; Pathak, Hsu & Arrighi, 1973). In different populations of a rodent, *Bandicota b. bengalensis*, variation due to deletion of constitutive heterochromatin in the short arm of the X chromosome has also been reported (Sharma & Raman, 1973). The present investigation with C-band staining of *M. dunni* chromosomes has shown that the minute but distinct autosomal second arms are heterochromatic. It is also demonstrated that the large size of the X and Y chromosomes is the result of accumulation of C-band positive constitutive heterochromatin. All these results strongly suggest that constitutive heterochromatin is very much associated with the karyotypic divergence of the taxa. The polymorphic condition of autosomes observed in various populations or within a population of *M. dunni* is most likely, as in *Peromyscus*, to be due to addition or deletion of constitutive heterochromatin in the short arms rather than due to inversions as postulated by Matthey & Petter.

Prior to the advent of modern techniques, whenever organisms exhibited a constant diploid number but a varying number of chromosome arms, it was generally concluded that the variation was due to pericentric inversions or translocations. The recent findings in *Peromyscus* and *Bandicota* and now in *Mus* have, however, shown that an entirely different mechanism, i.e. addition or deletion of constitutive heterochromatin, might also account for such variations. This mechanism results in the alteration of genome size, and seems to have played a dominant role in the karyotypic evolution of certain taxa.

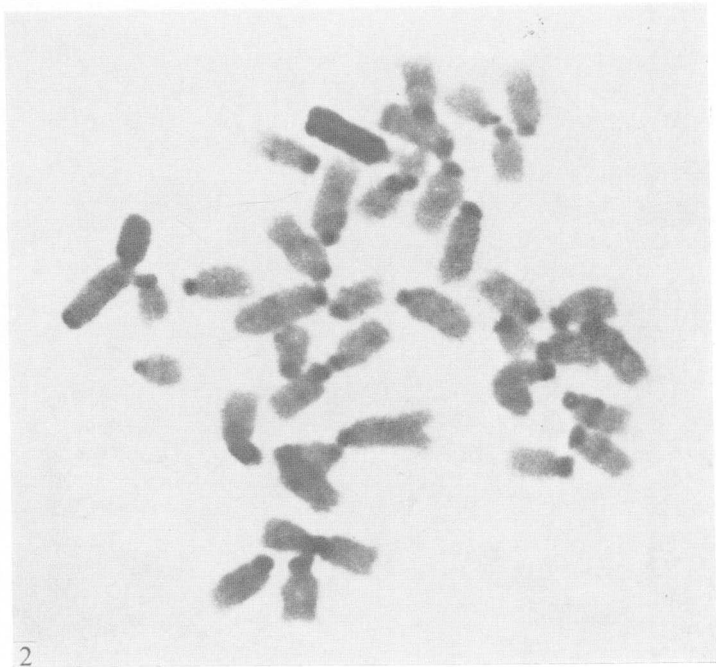
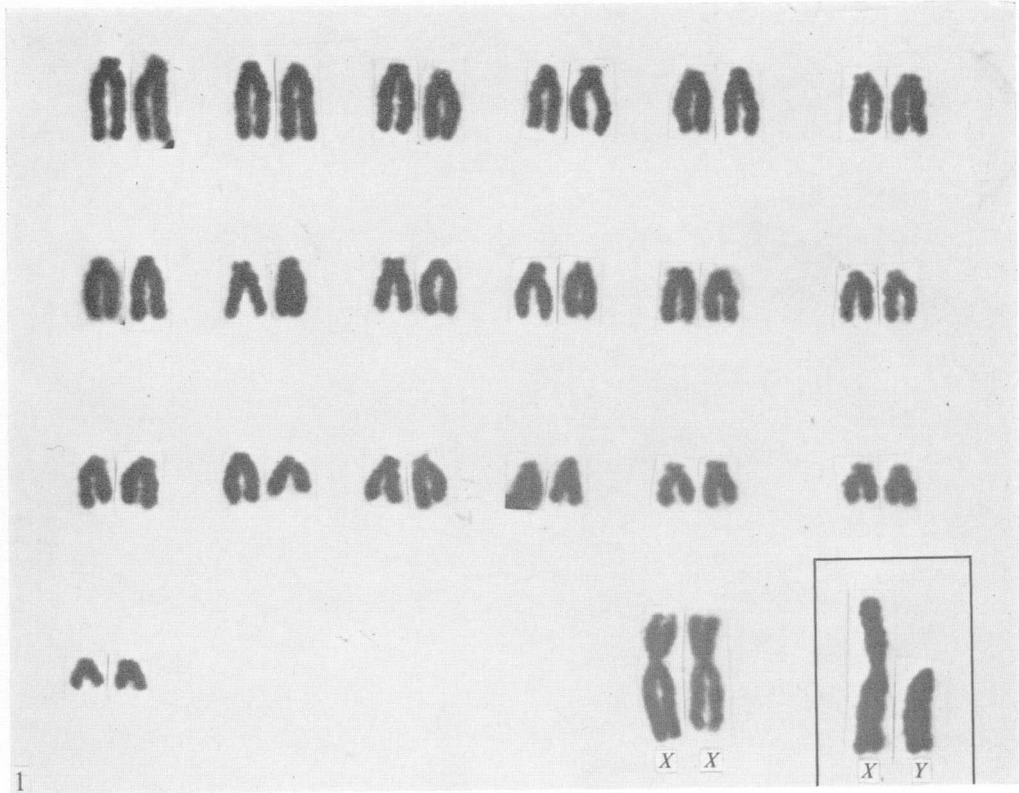


Fig. 1. Karyotype of a female *M. dunnii* with the X and Y chromosomes of a male in the inset. $\times 2000$.

Fig. 2. C-band staining of a male metaphase of *M. dunnii*. The autosomal short arms are stained deeply. The entire Y, except its centromeric region, and the complete short arm and the telomeric region of the long arm of the X are also deeply stained. $\times 2000$.

The nature of the influence of constitutive heterochromatin is, however, unknown at present. Investigations on these rodents would certainly help in understanding the evolutionary significance of constitutive heterochromatin.

We wish to thank Dr Joe T. Marshall for kindly identifying the specimens. Financial support from the University Grants Commission is gratefully acknowledged.

REFERENCES

- ARRIGHI, F. E. & HSU, T. C. (1971). Localization of heterochromatin in human chromosomes. *Cytogenetics* **10**, 81–86.
- BRADSHAW, W. N. & HSU, T. C. (1972). Chromosomes of *Peromyscus* (Rodentia, Cricetidae). III. Polymorphism in *Peromyscus maniculatus*. *Cytogenetics* **11**, 436–451.
- DUFFEY, P. A. (1972). Chromosome variation in *Peromyscus*: a new mechanism. *Science* **176**, 1333–1334.
- HSU, T. C. & ARRIGHI, F. E. (1966). Chromosomal evolution in the genus *Peromyscus* (Cricetidae, Rodentia). *Cytogenetics* **5**, 355–359.
- HSU, T. C. & ARRIGHI, F. E. (1968). Chromosomes of *Peromyscus* (Rodentia, Cricetidae). I. Evolutionary trends in 20 species. *Cytogenetics* **7**, 417–446.
- LEE, M. R., SCHMIDLY, D. J. & HUHEEY, C. C. (1972). Chromosomal variation in certain populations of *Peromyscus boylii* and its systematic implications. *Journal of Mammalogy* **53**, 697–707.
- MATTHEY, R. & PETTER, F. (1968). Existence de deux espèces distinctes, l'une chromosomiquement polymorphe, chez des *Mus* indiens du groupe *booduga*. Etude cytogénétique et taxonomique. *Revue Suisse de Zoologie* **75**, 461–498.
- PATHAK, S., HSU, T. C. & ARRIGHI, F. E. (1973). Chromosomes of *Peromyscus* (Rodentia, Cricetidae). IV. The role of heterochromatin in karyotypic evolution. *Cytogenetics & Cell Genetics* **12**, 315–326.
- SHARMA, T. & RAMAN, R. (1973). Variation of constitutive heterochromatin in the sex chromosomes of the rodent *Bandicota bengalensis bengalensis* (Gray). *Chromosoma* **41**, 75–84.
- SUMNER, A. T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* **75**, 304–306.