Somatic Chromosomes of Pangolin, Manis pentadactyla (Pholidota: Mammalia) with Special Reference to Satellite Association

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The diploid chromosome number of pangolin, Manis pentadactyla is a matter of controversy. Two renowned cytological schools reported different 2n numbers for the same species. Makino and Tateishi (1951) reported 2n=42 in a male pangolin, Manis pentadactyla collected from Japan. Ray-Chaudhuri et al. (1969) suggested 2n=36 for the same species collected from North-Eastern India. These two contradictory reports made us interested to investigate the chromosome complements of pangolin. In this report, we present a completely different 2n number for M. pentadactyla collected from two different North-East Indian states with notes on the formation of satellite association in this species.

Materials and methods

Four specimens (2♂ and 2♀) were collected in the environs of West Bengal and Tripura state between the months of June and November. Chromosomes were prepared from bone marrow and spleen by colchicine-hypotonic saline citrate-flame drying technique (Chakrabarti 1973, Chakrabarti and Chakrabarti 1977). C-band was performed by little modification (Chakrabarti and Chakrabarti 1978) of the technique prescribed by Sumner (1972).

Observations

An analysis of 20 well spread metaphase stage from each individual revealed 40 as the diploid number in both the sexes (Fig. 1). The karyotype consisted of 38 biarmed and a pair of small telocentric chromosomes (Fig. 2). The last 4 chromosomes bear satellites at their short arms. The X is a medium sized metacentric chromosome and Y is a small submetacentric element (Fig. 2). In our C-band preparation, the Y chromosome appeared to be wholly composed of heterochromatin and the X showed a large block of pericentromeric heterochromatin (Fig. 3).

Interesting is the satellite association in this species. In cells arrested during mid-metaphase stage, 3 out of 4 satellited chromosomes were locked in a close association with their satellited arms directed towards each other (Fig. 6). The association was so intimate that one can easily confuse this complex as a single unpaired...
Figs. 1–2. 1, metaphase spread from a female bone marrow cell (2n=40). 2, karyotype from a male metaphase.

subtelocentric chromosome. The other satellited member remained far apart from the association and usually occupied a lone peripheral position in most of the C-metaphases (Figs. 5 and 6). In prometaphase or in early metaphase stages all the 4 satellited members were separated from each other. A detailed survey of well
Figs. 3–6. 3, C-banded male metaphase showing uniformly C-positive Y chromosome. The broken arrow represents darkly stained pericentromeric heterochronatin of the X chromosome. 4 and 5, metaphase spreads from female (4) and male (5) bone marrow cells representing the initiation and progression of SA. 6, male metaphase showing close association between 3 SAT chromosomes; the broken arrow denotes the position of the other SAT chromosome.

spread metaphases at different stages of condensation revealed that, at the onset of satellite association, 2 of the 3 SAT chromosomes came closer with their satellite bearing arms opposed to each other (Fig. 4). The third member of this association perhaps trapped into the complex a little later (Fig. 5). Since no association was noted in cells arrested during late metaphase, we assume that the association disrupted at the end of metaphase and the associated members dispersed for anaphase separation.

Discussion

Previously, Makino and Tateishi (1951) reported $2n=42$ for a male pangolin collected from Japan. Ray-Chaudhuri et al. (1969) reported $2n=36$ for the same
species from a single male specimen captured in a village of North-East India. Matthey (1970) supported the report of Ray-Chaudhuri et al. (1969). The report published by Makino and Tateishi (1951) was about 30 years ago. Since at that time colchicine-hypotonic treatment technique was not applied, it was really very difficult to determine the morphology and exact number of chromosomes from a somewhat clumped chromatin mass. When communicated, Prof. Makino personally wrote to me “the variation noted may be due to technical shortcomings” because “our study based on paraffin sectioning method”.

In their report Ray-Chaudhuri et al. (1969) arbitrarily designated a subtelo-centric chromosome as the X chromosome. We could not confirm their findings because, in our material the X seems to be a medium sized metacentric chromosome. Interestingly, the satellite association between 3 small chromosomes during mid metaphase exhibited a unique resemblance to the X chromosome designated in the report of Ray-Chaudhuri et al. (1969). Again, the Y chromosome designated in the paper of Ray-Chaudhuri et al. (1969) appeared to be a small acrocentric element; this in our material is apparently identical with the fourth member of the satellite bearing chromosome which showed a lone peripheral disposition, often far away from the remaining 3 associated members (Fig. 6). The reported occurrence of 3 different 2n numbers (including this report) for the same species, *M. pentadactyla*, may lead one to assume the possibility of chromosome polymorphism in this particular species. But we are not prepared to consider this as a case of chromosome polymorphism. Because, unlike our previous investigators, we studied both male and female specimens collected from two widely separated Indian states and metaphase spreads from both spleen and bone marrow from all the specimens revealed 40 as the diploid number in this species.

The formation of satellite association (SA) has often been attributed to the involvement of SAT chromosomes in the formation of the nucleolus (Hanssen 1970). Hsu (1965) suggested that the sticky nucleolar materials hold the associated chromosomes through mitosis. In pangolin the mode of formation of SA is interesting. Because in the prometaphase no association was noted; but in the cells arrested at mid-metaphase stage, the association was very prominent. Thus the mode of formation of SA in pangolin and the postulated significance of the phenomenon (Hsu 1965) indicate the possibility of the existence of nucleolar material in the cells even during the metaphase stage of cell division. A similar generalised idea was also advocated earlier by Hsu (1965) and Ghosh (1976). The non-involvement of one SAT chromosome in association may be due to the loss of nucleolus organizing region (NOR) in the short arm of this particular chromosome.

**Summary**

Somatic chromosome complements of pangolin, *Manis pentadactyla* have been studied from both male and female specimens collected from two widely separated states of India. The 2n number is 40 (neither 42 nor 36 as reported earlier) in both sexes and the NF (determined on the basis of the number of major autosomal arms) is 64. The X is a medium sized metacentric chromosome and the Y is a small
C-band positive submetacentric element.

Temporary satellite association between 3, out of 4 small satellite bearing chromosomes has been noticed during metaphase.

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References

Makino, S. 1980. Personal communication to the first author.

Note added in the proof

In a recent communication to the first author Prof. Ray-Chaudhuri told that the specimen they studied may be a different species. Our discussions were based on Ray-Chandhuri at al. (1969).