Chromosomes and the Karyotype of the Pangolin, *Manis pentadactyla* L. (Pholidota-Mammalia)

The chromosomes of higher eutherian mammals are now fairly well known but the same is not true for lower eutherian mammals, such as Edentata and Pholidota. Prior to the introduction of current cytological techniques, MAKINO and TATEISHI[1] reported $2n = 42$ chromosomes in a male Pangolin, *Manis pentadactyla* belonging to the order Pholidota from Japan. During the course of chromosome studies in several mammalian species, we were able to obtain a male Pangolin which was identified as *Manis pentadactyla* L., from the Chakia Forest, a place nearly 30 miles away from Varanasi in Uttar Pradesh (India). Although our material is apparently identical with that of MAKINO and TATEISHI[1], yet our results are entirely different from theirs in chromosome number as well as in morphology. We are therefore reporting our findings on the chromosomes of *M. pentadactyla* from India, using Colcemid pretreated bone marrow cells. The usual air-drying technique was adopted in preparing the slides which were stained in Unna Blue.

Results. After studying 126 metaphase plates, the diploid chromosome number was determined to be 36 (Figure 1) as against 42 reported earlier (MAKINO and TATEISHI[1]) from spermatogonial cells. The frequency distribution of the chromosome numbers encountered in the present investigation is shown in the Table. The whole chromosome complement may be divided into 4 distinct groups (Figure 2).

The first group includes 7 pairs (1–7) of large to small sized metacentric autosomes. Only pair No. 1 in this group can easily be identified, because it is the longest metacentric chromosome pair. There are 6 pairs (8–13) of medium-sized submetacentric chromosomes in the second group. Pair Nos. 8 and 9, because of their similar morphology, cannot be distinguished one from the other. Pairs 10–13 are more or less of the same relative length and so they cannot be separated from each other. The X chromosome also belongs to this (10–13) group. The 3rd group includes 3 pairs (14–16) of distinguishable large-sized subtelocentric autosomes. Pair No. 14 is the largest pair in the complement and may be easily identified. Pairs Nos. 15 and 16 are not distinguishable from each other. One of the homologues of the pair No. 16 sometimes shows a very clear achromatic gap in the long arm. But this achromatic gap was not observed in all the metaphase plates examined and therefore the gap cannot be used as a 'marker' for this pair. A pair of smallest-sized acrocentric autosomes constitutes the 4th group. Pair No. 17 can be very easily identified in all metaphase plates.

Since the individual was a male and no female was available the X chromosome has been identified arbitrarily. As has been stated earlier the X is a medium-sized subtelocentric chromosome. The Y is the smallest acrocentric chromosome in the male set. Figure 3 shows the idiogram prepared for this species.

The testis preparation did not reveal many dividing cells. Perhaps the animal was captured in an unfavourable season. Blood leucocyte culture also failed to reveal any dividing plates.

Discussion. The only report of chromosome study of Pholidota comes from MAKINO and TATEISHI[1] who described $2n = 42$ chromosomes in a male Pangolin, *M. pentadactyla*. The X chromosome was reported to be the largest-sized, J-shaped subterminal chromosome and the Y was a little larger rod-shaped element than the smallest autosome pair. We could not, however, confirm their findings. In a personal communication MAKINO[2] writes: 'Data from classical methods should be revised by current techniques.'

The chromosome number as revealed from our study is $2n = 36$ and the X is a medium-sized submetacentric chromosome and the Y, an acrocentric chromosome, is

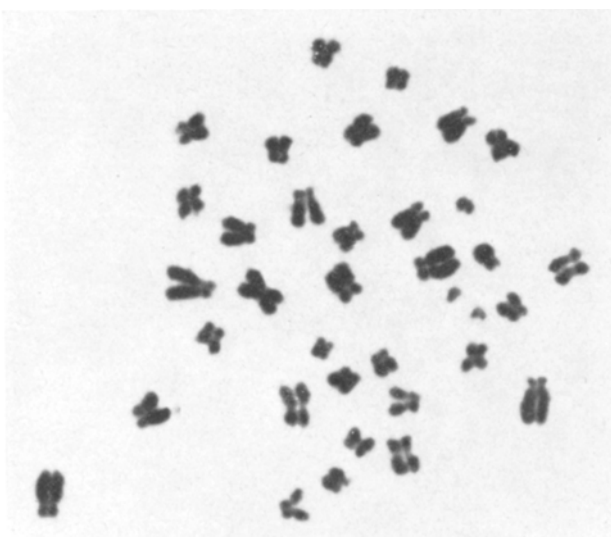

Fig. 1. Male metaphase plate of *M. pentadactyla*. × 2200.

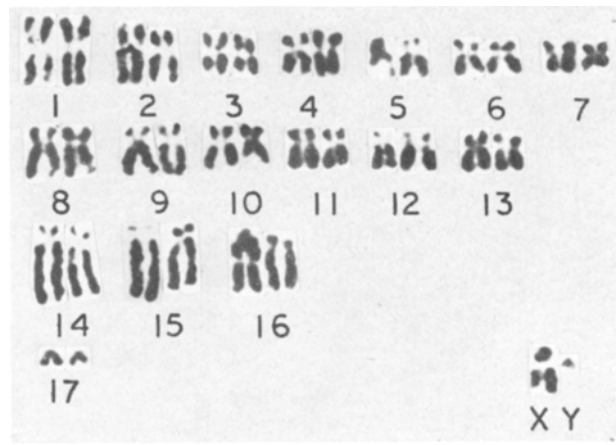

Fig. 2. Karyotype of metaphase of male *M. pentadactyla*. × 2200.

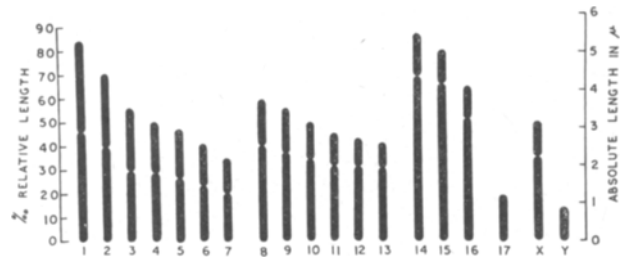

Fig. 3. Idiogram of *M. pentadactyla*.

[1] S. MAKINO and S. TATEISHI, J. Fac. Sci. Hokkaido Univ. VI, Zool. *10*, 319 (1951).

[2] S. MAKINO, personal communication (1968).

smaller than the last pair of autosomes (Figure 3). The number of chromosome arms (FN) is determined to be 66, excluding the sex chromosomes.

The separation of Pangolins from Edentata into a separate order Pholidota which is entirely based on the anatomical features, is further supported by cytological findings also. In the family Dasypodidae of Edentata, the diploid chromosome number ranges from $2n = 58$ in *Euphractus*, 60 in *Chaetophractus* to 64 in *Dasypus* (see MATTHEY[3]). In *D. novemcinctus*, where the karyotype has been studied in greater detail, the number of chromosome arms is 78 (BEATH et al.[4]), of which 23 pairs are acrocentrics whereas in M. *pentadactyla* only 1 pair of smallest sized autosomes is acrocentric. The karyotype of the family Dasypodidae is characterized not only by a larger number of chromosomes but also by a higher number of chromosome arms than Manidae. The karyotype of *M. pentadactyla* ($2n = 36$, FN = 66) is quite different from the karyotypes of the genera belonging to the family Dasypodidae. Thus, the inclusion of Pangolins in a separate order Pholidota is fully justified.

It is the authors' desire to explore, if possible, the 4 African and the 2 remaining Asiatic species of the genus *Manis* cytologically.

Chromosome counts in the marrow cells of a Pangolin

Sex	Chromosome number					Total cells counted
	33	34	35	36	<	
Male	1	7	7	107	4[a]	126

[a] Polyploid cells.

Zusammenfassung. In Knochenmarkzellen wurden die Chromosomen eines männlichen Pangolins (*Manis pentadactyla* L.) untersucht. Die Zahl der diploiden Chromosomen beträgt 36. Dieser zytologische Befund unterstützt die Ansicht, dass man die Pangoline in eine besondere Ordnung der Pholidota einreiht.

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[3] R. MATTHEY, Mammalian Chromosomes Newsl. *20*, 47 (1966).
[4] M. M. BEATH, K. BENIRSCHKE and L. E. BROWNHILL, Chromosoma *13*, 27 (1962).

Initiation of Mitosis in Interphase Plasmodia of *Physarum polycephalum* by Coalescence with Premitotic Plasmodia[1]

The plasmodia of the myxomycete, *Physarum polycephalum* coalesce readily with one another upon contact[2]. If coalescence occurs between plasmodia representing different stages of the mitotic cycle[3], the nuclei in the resulting mixed plasmodium enter the next postfusion mitosis in synchrony[4,5]. The time which elapses between fusion and the onset of this mitosis depends on the proportion in which the constituents from plasmodia of different stages of the mitotic cycle are present. If this proportion is such that nuclei of two different stages of the mitotic cycle are present in about equal numbers, the first synchronous mitosis after fusion occurs at a time which is about half way between the times at which the next division takes place in control pieces which were set aside from the same plasmodia prior to fusion[4-6]. In previous experiments it was found that premitotic plasmodia in such combinations had a slightly dominant effect, i.e., if they were allowed to coalesce with postmitotic plasmodia, the nuclei of the resulting mixed plasmodia divided somewhat earlier than would be expected from the proportion in which the nuclei of both stages were present[6].

This dominance is considerably more pronounced in combinations of premitotic plasmodia with interphase plasmodia which were maintained on non-nutrient balanced salt solution[7] for about a week before the experiment. In such starved plasmodia, the nuclei continue to divide in synchrony[8], although the duration of the intermitotic period is considerably prolonged, and a number of nuclei degenerate between mitoses. At all stages of the mitotic cycle, the nuclei of starved plasmodia are considerably smaller than nuclei from growing plasmodia (Figures 1 and 2). Thus, both types of nuclei can be readily distinguished from one another, under phase contrast, in ethanol-fixed smear preparations from plasmodial explants containing a mixture of both types of nuclei.

In the fusion experiments reported below, the premitotic plasmodia at the time of coalescence were at a stage approximately 35 min prior to metaphase. At this time, the central nucleolus within each nucleus is located closer to the nuclear membrane than is the case in interphase nuclei of an earlier stage. The chromosomes have begun to withdraw from the nuclear membrane and are accumulating on one side of the nucleolus[3]. Segments of these plasmodia were allowed to coalesce, by sandwiching[9], with segments from interphase plasmodia which had been starved for a period of 1 week preceding the experiment. As in previous experiments[10], other segments from both premitotic and interphase plasmodia were set

[1] Supported by AEC contract No. COO-1432-9.
[2] E. GUTTES and S. GUTTES, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), vol. 1, p. 43.
[3] E. GUTTES, S. GUTTES and H. P. RUSCH, Devl Biol. *3*, 588 (1961).
[4] E. GUTTES, S. GUTTES and H. P. RUSCH, Proc. Fedn Am. Soc. exp. Biol. *18*, 479 (1959).
[5] H. P. RUSCH, W. SACHSENMAIER, K. BEHRENS and V. GRUTER, J. Cell Biol. *31*, 204 (1966).
[6] E. GUTTES, V. R. DEVI and S. GUTTES, Experientia *25*, 615 (1969).
[7] J. W. DANIEL and H. H. BALDWIN, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), vol. 1, p. 9.
[8] E. GUTTES and S. GUTTES, Proc. Fedn Am. Soc. exp. Biol. *20*, 419b (1961).
[9] E. GUTTES and S. GUTTES, Experientia *23*, 713 (1967).
[10] S. GUTTES and E. GUTTES, J. Cell Biol. *37*, 761 (1968).