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.

Chromosome counts in the marrow cells of a Pangolin

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

[a] Polyploid cells.

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

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

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[1] Supported by AEC contract No. COO-1432-9.

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[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).

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[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).

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[10] S. GUTTES and E. GUTTES, J. Cell Biol. *37*, 761 (1968).

aside, prior to fusion, to serve as controls. The time of mitosis in the controls as well as in the mixed plasmodia was determined as described previously[10].

The results of the present experiments, in those combinations in which coalescence occurred in time before the premitotic nuclei had entered mitosis, fell into 2 groups. In one group, the nuclei from the starved interphase plasmodia entered prophase, in the mixed plasmodia, along with the nuclei from the premitotic plasmodia, within approximately 40 min after coalescence (Figure 3). This stage in starved plasmodia[11] is characterized by condensation of the chromosomes around the nucleolus (Figure 4), whereas in growing plasmodia the nucleolus during the same stage is located on one side of the condensed mass of chromosomes (Figure 3).

Initiation of mitosis in interphase nuclei within mixed plasmodia partially composed of constituents from premitotic plasmodia was observed as early as approximately

Fig. 1. Nuclei of growing plasmodium, approximately 2 h before mitosis (duration of intermitotic period in this plasmodium 10.5 h). Note large central nucleolus. Phase contrast; ethanol-fixed smear preparation (same for the Figures 2–4). × 5750.

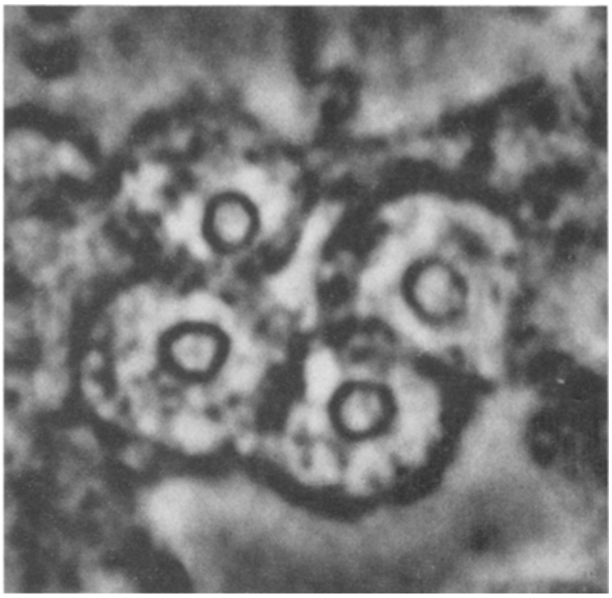

Fig. 2. Nuclei of starved plasmodium, interphase, approximately 12 h before mitosis. The intermitotic period in this plasmodium lasted a little less than 3 days. × 5750.

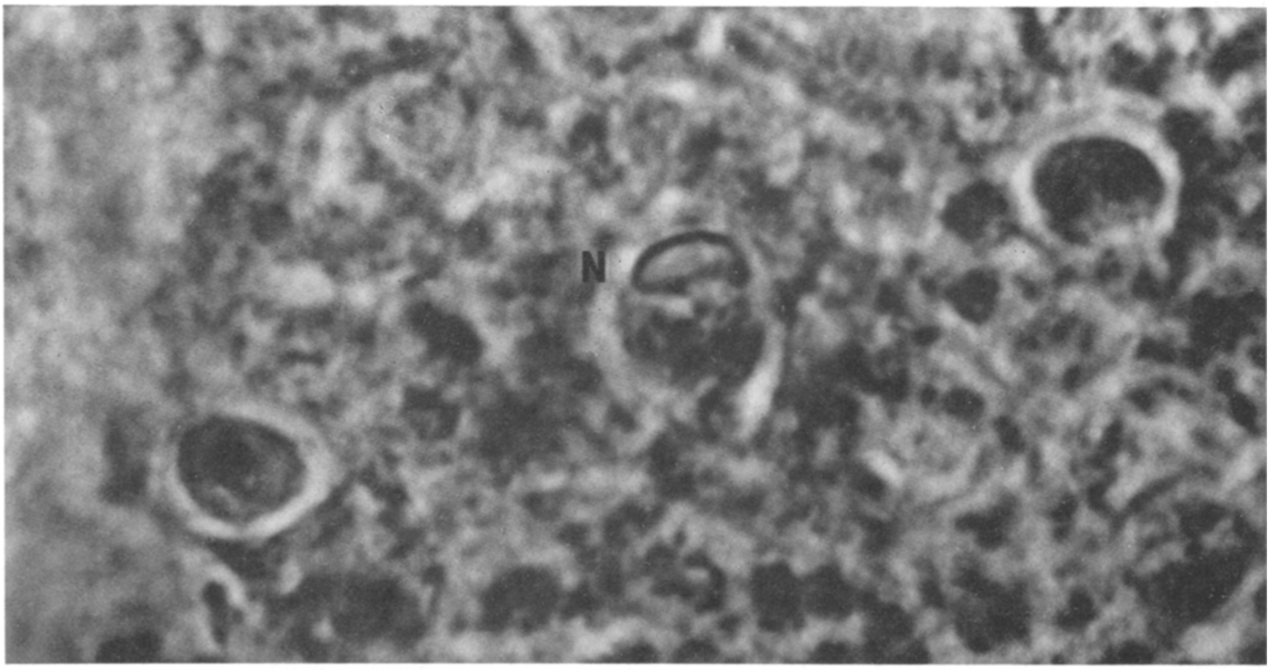

Fig. 3. Nuclei in a mixed plasmodium approximately 30 min after coalescence. All nuclei are in early prophase. N, nucleus from premitotic plasmodium; the 2 other nuclei are from starved interphase plasmodium. × 4500.

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[11] E. Guttes and S. Guttes, Experientia *20*, 269 (1964).

12 h prior to the mitosis in the starved controls, and in combinations in which the number of interphase nuclei within the mixed plasmodia was up to 3.6 times that of the nuclei from the premitotic plasmodia; e.g. in the experiment shown in Figure 3, the proportion in the mixed plasmodium of interphase nuclei to premitotic nuclei was between 2.4/1 and 3.6/1, as determined in smear preparations of explants taken from different parts of the plasmodium, and the interphase nuclei in the mixed plasmodium entered prophase, along with the nuclei from the premitotic plasmodium, approximately 30 min after the beginning of prophase in the premitotic control and 12.4 h before the beginning of mitosis in the starved interphase control.

Once initiated, mitosis in the mixed plasmodia went to completion in most of the nuclei. In the nuclei which did not divide, the nucleoli failed to disintegrate during prophase, and chromosomes and nucleoli clumped in the middle of the nuclei which became pycnotic.

The onset of prophase within the mixed plasmodia, without considerable delay as compared to the premitotic controls and in a number of interphase nuclei more than twice that of the premitotic nuclei present, suggests that prophase was initiated by a diffusible factor(s) which was either present in the premitotic plasmodia at the time of coalescence or which was produced, after coalescence, under the influence of constituents, including nuclei, that were contributed by the premitotic plasmodia.

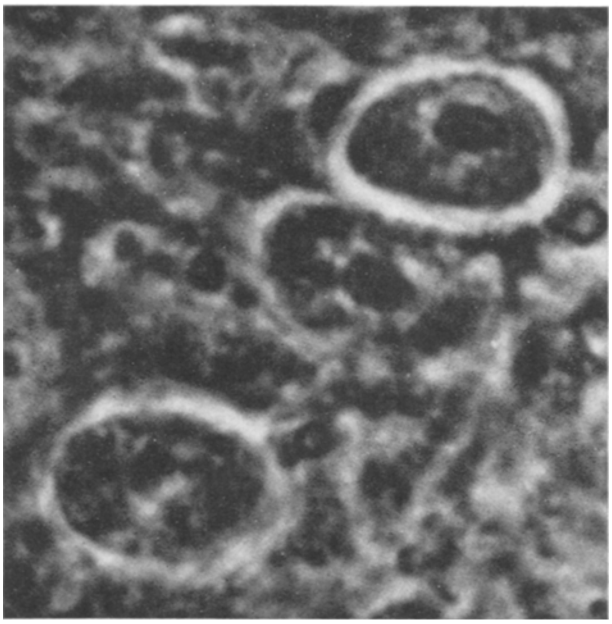

Fig. 4. Nuclei of starved plasmodium, early prophase. Nucleoli surrounded by condensed mass of chromosomes. × 5750.

In a number of other, seemingly similar combinations, no such initiation of mitosis was observed, and the interphase nuclei which were contributed by the starved plasmodia showed no morphological change in the presence of the premitotic nuclei. The morphological appearance of the latter gradually reverted to that of interphase nuclei (nucleolus in the middle of the nucleus, chromosomes evenly distributed in proximity to the nuclear membrane), and they decreased in size until, approximately 8–10 h later, they were no longer distinguishable from the interphase nuclei that were contributed by the starved plasmodium. In this case, observation was discontinued approximately 12 h after coalescence. This negative result could have been due to an extremely short duration of the initiating state in the premitotic plasmodia and the resulting difficulty in achieving coalescence at the right time.

Attempts to initiate mitosis, also by sandwiching, by fusion of premitotic plasmodia with growing, rather than starved, plasmodia, were unsuccessful. It would appear, therefore, that in starved plasmodia, but not in growing plasmodia, mitosis is preceded by a sufficiently long period during which an initiating factor(s) is the last remaining requirement for the beginning of prophase. Starved plasmodia, therefore, during the latter part of their intermitotic period, or plasmodia which are maintained on deficient, defined medium, would provide suitable indicator systems to determine the presence of mitosis initiating factors either in vivo or in subcellular fractions.

Zusammenfassung. Es wird gezeigt, dass eine Kernteilung innerhalb sehr kurzer Zeit eintritt, wenn Teile eines Plasmodiums von *Physarum polycephalum*, das zur Teilung ansetzt, mit Teilen von Plasmodien verschmolzen werden, die sich in Ruhe befinden. Daraus wird auf eine stoffliche Induktion zur Mitose geschlossen.

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Monocentric Nature of the Chromosomes of *Ranatra* (Heteroptera) Verified by the Induced Fragmentation Experiments

Recently from our observations on the morphology and the anaphase configurations of the chromosomes of a heteropteran insect *Ranatra elongata*, we (DESAI and DESHPANDE[1]) have proposed that the chromosomes of this insect are monocentric and not polycentric as is generally believed. The one sure test to demonstrate the polycentric nature of the chromosomes is to induce fragmentations in them by irradiation techniques and see if the fragments survive as independent chromosomes during cell divisions. In the present work, results of such experiments on adult males of *Ranatra filiformis* Fabr. having the chromosome number $n = 23$ $(19A + 3X + 1Y)$ are described. Groups of 10 were irradiated at various dosages as 500r, 800r, 1000r, 2000r, 3000r, 5000r, 8000r, 10,000r, 12,000r and 15,000r, using the source 'Gamma cell 200' at the rate of 4.45 Kr/min. Owing to their extremely small size, these chromosomes could be fragmented only at the dosages of 5000r and above. Dosages up to 10,000r induced fragmentations considerably, and hence numerous cells showed anaphase bridges. Yet the

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[1] R. N. DESAI and S. B. DESHPANDE, Experientia *25*, 384 (1969).