

CYTOLOGICAL STUDIES IN HYMENOPTERA.
III. CYTOLOGY OF PARTHENOGENESIS IN THE FORMICID
ANT, *CAMPONOTUS COMPRESSUS*

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INTRODUCTION

Due to the parthenogenetic development of the male hymenoptera, the study of the male — female chromosomal relationship, nature of the male meiosis I and the mechanism of sex-determination assumes useful significance. The present cytological findings on the common Indian formicid ant, *Camponotus compressus*, are in continuation of the the author's earlier work on the cytology of the honey-bees, *Apis indica* (SHARMA *et al.*, 1961) and *A. dorsata* (KUMBKARNI 1964), and the carpenter-bee, *Xylocopa fenesterrata* (KUMBKARNI 1965a).

Haploid male — diploid female chromosomal constitution is 10/20. The presence of only a single genome derived from the mother obliterates the formation of tetrad formation, typical of the meiotic prophase in the male meiosis I, which is completely abortive: there is not even a trace of any spindle formation, chromosomes simply clumping into a single mass within the intact nucleus, and no cytoplasmic bud simulating the cytokinetic activity is pinched off as in the bees. Unlike the latter the second meiotic division has equational cytokinesis producing two equal functional spermatids. The chromosomes have a general tendency to aggregate to the extent of almost clumping at times. As in the honey-bee, one chromosome lags behind for only short time during the anaphase of male meiosis II; however no sex-determining significance can be ascribed to it (eg. KUMBKARNI 1965b), as has been suggested for the honey-bee by MANNING (1949). Till the genetics of this insect is worked out, « multiple sex-allele theory », applied to *Apis mellifica* by ROTHENBUTLER (1958) and others, can be considered to hold good in explaining the sex-determination in the present case also. Wherever necessary the observations were confirmed by studying the living cells under the phase-contrast microscope.

MATERIAL AND METHODS

The spermatogenesis is completed just when the pigmentation of the pupae starts changing from white to brown. Testicular material from the late larvae, pre-pupae and pupae gave good results for studying the spermatogonial metaphases, abortive meiosis I and meiosis II respectively. Pupal ovaries yielded reliable oogonial counts. For ascertaining the somatic chromosomal determinations of both the sexes, the dividing larval cerebral ganglionic cells and the early cleavage cells have been studied.

Though practically all the current standard techniques were employed to study the chromosomes, Sanfelice and Carnoy fixed/iron haematoxylin preparations produced quite good results. In addition squashes of acetyl alcohol fixed/aceto-carmine stained material were prepared for determining the spermatogonial, oogonial and the somatic complements. The chromosomal nature of the lagging element in the male meiotic anaphase was confirmed with the Feulgen's nucleal reaction.

Wherever necessary living cells were also studied to confirm or supplement the data from the fixed preparations with Carl-Zeiss stand «W» microscope having Achromatic 1.25 phase-contrast condenser, PH 1.25/100 phase-contrast objective with a phase plate having 65 P.C. absorption, and K8 \times ocular.

OBSERVATIONS

Haplo-diploid chromosomal relationship.

Oogonial and spermatogonial polar-view counts reveal 20 and 10 chromosomes respectively (Figs. 5, 6). Dividing larval cerebral ganglionic cells (Figs. 1, 2) and the cleavage cells (Figs. 3, 4) of the female and male have also been observed to possess the same numbers of 20 and 10 chromosomes

Figs. 1-16. — *Camponotus compressus*.

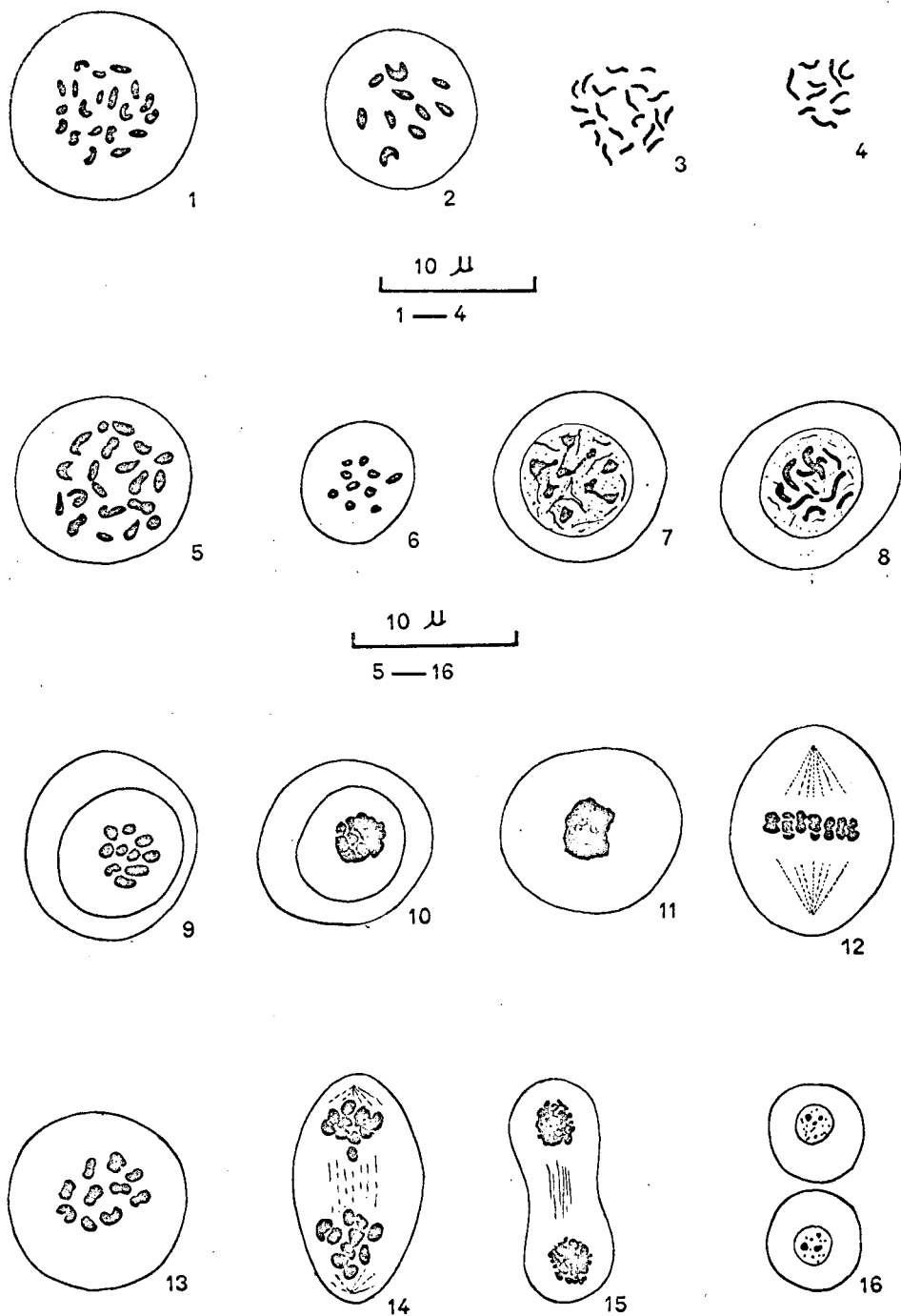
Figs. 1-2. — Metaphase polar-views showing the diploid and haploid numbers of 20 and 10 chromosomes each from the female and male dividing larval cerebral ganglionic cells.

Figs. 3-4. — Somatic determinations of 20 and 10 from the cleavage cells of the female and male respectively.

Figs. 5-6. — Oogonial and spermatogonial metaphase polar view counts of 20 and 10 chromosomes each.

Figs. 7-11. — Abortive male meiosis I: Figs. 7-8. Prophase stages; Fig. 9. Haploid set of 10 fully condensed chromosomes lies in the nucleoplasm, in the absence of any spindle to attach with. The nuclear area has increased; Figs. 10-11. The chromosomes soon clump into a single mass, which brings an end to male meiosis I. The nuclear membrane disappears.

Figs. 12-16. — Male meiosis II: Fig. 12. Metaphase—there is no discernible prophase II preceding the appearance of metaphase II chromosomes; the spindle present is the only one formed during the spermatogenesis; Fig. 13. Metaphase polar-view giving the same count of 10 chromosomes as present in the primary spermatocyte; Fig. 14. Late anaphase. One chromosome at the upper pole lags behind, though quite inconspicuously, for a very short time; Figs. 15, 16. Cytoplasmic cleavage is equal and 2 functional spermatids are formed.



each. The chromosomes of the cleavage-cells are long and slender. The somatic chromosomes of the female lie more or less in pairs, which points to the fact that 10 unpaired discrete chromosomes of the male constitute a single genome. The chromosomes so closely resemble each other that even in the female it is difficult to distinguish the different pairs.

Cytology of spermatogenesis.

Abortive meiosis I. — At the onset of prophase the hitherto diffused and scattered chromatin, accompanied by an increase in the nuclear volume, gets transformed into single chromosomal threads. A slight shortening and thickening of the latter result in the formation of 10 discrete chromosomes (Figs. 7-9). Neither the extra nor the intra-nuclear spindle is present. The nuclear membrane, however, persists. Thus in the absence of any spindle to associate with, the chromosomes at a stage, otherwise comparable to the metaphase, lie near each other, as such, for sometime in the centre of the nucleus (Fig. 9). Soon they come very close to one another and gradually clump together into a single mass, thereby losing their visible individual entity (Figs. 10, 11). No cytoplasmic bud (vestigial cytokinesis) is produced, and that brings to an end the meiosis I, the only evidence of which is the incomplete intra-nuclear activity resulting in the formation of a haploid set of 10 chromosomes. The absence of the cytoplasmic bud and the clumping of the chromosomes were also verified by studying the living cells under the phase-contrast microscope.

Meiosis II. — Without any apparent change in the chromosomal mass of meiosis I, 10 diads (Fig. 13) separate and arrange themselves on the equator of a freshly formed bipolar spindle as soon as the nuclear membrane disappears (Figs. 11, 12). It may be remarked that it is the only spindle met with throughout the male meiosis. During anaphase 10 chromosomes move to each pole. One of these, however, shows a slight tendency to lag quite inconspicuously rather late in the anaphase (Fig. 14), but ultimately it too joins the respective complement at the telophase (Fig. 15). A normal mitotic division is thus accomplished, resulting in the formation of two equal functional spermatids (Fig. 16).

DISCUSSION

Haplo-diploidy and chromosomal constitution.

Haplo-diploid relationship of the formicid ant, *Componotus compressus*, has been established by the presence of a single genome of 10 chromosomes in the male and a double genome of 20 chromosomes in the female. Somatic determinations do not suggest any polyploidy, reported in some other hymenoptera by others.

LAMS (1908) confesses that he could not ascertain the haploid number for *C. herculeanus*, though his figures suggest a complement of 14, 15 or 16 chromosomes in the primary spermatocyte. However, he considers 32 as the diploid number from the female. In view of such a great difference in the number of chromosomes of *C. compressus* of the present investigations and *C. herculeanus* of LAMS, a fresh enquiry of the latter is imperative.

WHELDEN and HASKINS (1953) consider 5 as the haploid number for all the 19 formicid ants studied by them. Such a view seems improbable since their material, comprising many highly divergent species, is very much heterogeneous. The present author feels that due to the small size of the chromosomes and their tendency to aggregate closely, as also indicated by the present work, an error has been made by WHELDEN and HASKINS in arriving at this haploid number.

SMITH and PEACOCK (1957) have found 11 and 22 as the haploid and diploid numbers for the formicid ant, *Monomorium pharaonis*, sub-family Myrmicinae.

Only the diploid numbers have been reported for the following formicids (sub-family, Formicinae): *Lasius niger*, 20 (HENKING 1892); *L. flava*, 24 (HOGBEN 1920); *Formica sanguina*, 48 (SCHLEIP 1908).

Keeping all these chromosomal constitutions in view, SMITH and PEACOCK (1957) postulate 11 or thereby as the basic haploid number of the family Formicidae, *Camponotus herculeanus* and *Formica sanguina* being roughly triploid and tetraploid. In that case the haploid number of 10 in the present species is nearer the presumed basic number of 11 for the family as a whole.

Cytology of spermatogenesis.

Abortive meiosis I. — The existence of an abortive first meiotic division in the ants, in accordance with other arrhenotokous hymenoptera, was noticed for the first time in *Camponotus herculeanus* by LAMS (1908). But his information is brief and incomplete.

WHELDEN and HASKINS (1953) have given a very improbable account of spermatogenesis in 19 formicid ants. According to them, the usual pattern of meiosis in the males of hymenoptera is that « the first meiotic division is equational, the second reductional; four functional sperms come from each primary spermatocyte ». Strange as it would appear none of these two divisions has been described as abortive, though their drawings (number 9 on plate I) seem to be suggestive of such a division. The common mode of male meiosis in hymenoptera is quite the reverse of what WHELDEN and HASKINS have stated and can be summarised as: the primary spermatocyte passes through an abortive first meiotic division, which is otherwise a reductional division in the males of zygogenetic groups, to become a « secondary spermatocyte », which

in turn undergoes the usual equational second meiotic division. The only reported exception is *Telenomus fariai* (DREYFUS and BREUER 1944), a highly specialised parasitic scelionid wasp far removed systematically from the social ants, where it is the second meiotic division which is not consummated. However, even in this instance an abortive division, though it is the second, denied by WHELDEN and HASKINS, is definitely demonstrated. The only similarity is the claimed equational first meiotic division. It is rather astonishing that WHELDEN and HASKINS do not even refer to this work in their paper.

Quite recently SMITH and PEACOCK (1957) have shown an abortive first division and an equational second division in the Pharaoh's ant, *Monomorium pharaonis*.

Likewise in the present work it has been established that the abortive division is the first, while the second is equational. Thus the views of WHELDEN and HASKINS are considered as mistaken.

In connection with the abortive meiosis I of the ants, *Monomorium pharaonis*, (SMITH and PEACOCK 1957) and *Camponotus herculeanus* (LAMS 1908), a cytoplasmic bud is reported to be detached as in *Apis* and some other hymenoptera. But it is important to point out that no such bud, considered to be the rudimentary secondary spermatocyte wherever present, is pinched off in the present material, as has been already observed by the author in the carpenter-bee, *Xylocopa fenesterrata* (KUMBKARNI 1965a), the wasp, *Polistes hebraeus*, and the ichneumon, *Polysphincta ceylonica* (KUMBKARNI 1961). Similarly neither any extra- nor intra-nuclear spindle fibres of the bees are present. The latter are not even represented by the probable fibres or striations, stretching from the chromosomes to the nuclear membrane, as noted by SMITH and PEACOCK.

It is quite apparent that the abortion of the male meiosis I in the present case has progressed so far that the latter is represented only by the appearance of 10 chromosomes which soon clump in to a single mass within the intact nucleus, a condition which on a cursory study may wrongly suggest that the first meiotic division is wanting at all. It is interesting to point out that the evidence of male meiosis I is better preserved in some of the hymenoptera taxonomically lower to the present material, e.g. the braconid, *Bracon nicevillei*, the ichneumon, *Polysphincta ceylonica*, and the saw-fly, *Athalia proxima* (KUMBKARNI 1961). Such observations tend to support the view that the evolution of the male haploidy in the hymenoptera had probably a multiple origin.

The consideration of SMITH and PEACOCK that the usual clumping of the chromosomes at the culmination of the first abortive meiotic division in the ants and other hymenoptera is an artefact is not acceptable. These authors have further claimed that the chromosomes at such a stage retain their identity, as has been maintained earlier by SANDERSON and HALL (1948) for *Apis mellifica*. However, the existence of this clumping, as a persistent and genuine feature,

has been confirmed by studying the living cells also under the phase-contrast microscope during present investigations and in *Apis indica* (SHARMA, GUPTA and KUMBKARNI 1961). The chromosomes mostly lose all visible individual identity.

Second meiotic division. — SMITH and PEACOCK (*see above*) have reported that in 1-2 percent of the cases, the second spermatocyte division in *Monomorium pharaonis* is unequal regarding the cytoplasm with the result that one of the two spermatid nuclei receives very little of cytoplasm: a phenomenon of regular occurrence in Apidae, noted by the author and others. But in the present case the second meiotic division is always equal, producing two equal functional spermatids. However viewed from that fact that the ants are taxonomically very close to the apids, this sporadic anomaly in *Monomorium pharaonis* suggests the evolutionary gradation by which a definitive and regular condition might have been arrived at in the apids.

One chromosome lags behind the rest quite late in the anaphase, though only for a short time (cf. single Feulgen positive isolated body in the first spermatocyte division of *M. pharaonis*, reported by SMITH and PEACOCK). This chromosome can be compared to the lagging chromosome in *Apis dorsata* (KUMBKARNI 1964) and *A. mellifica* (MANNING 1949); however it has no sex-determining significance as suggested by MANNING (KUMBKARNI 1965b).

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SUMMARY

1. In accordance with other Hymenoptera, the male-female chromosomeal relationship has been found to be haplo-diploid, viz. 10/20, in the formicid ant, *Camponotus compressus*.
2. There being only a haploid set of chromosomes in the male, the meiosis I is abortive: the only evidence of this 'division' is the intra-nuclear appearance of erstwhile metaphase chromosomes.
3. Unlike the honey-bee, the male meiosis II produces two instead of one functional spermatids.