Cytological Studies in Hymenoptera : Part II: Cytology of parthenogenesis in the carpenter-bee, *Xylocopa fenestata* Fabre

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Introduction

In all the Hymenoptera studied so far, males develop from unfertilised eggs and females develop from the fertilised eggs (arrhenotokous parthenogenesis). The consequent vital implications involved, due to the presence of only a single set of maternal chromosomes in the male, are mainly: (1) the viability of the haploid male; (2) the haploid male/diploid female chromosomal relationship; (3) the nature and significance of the first spermatocyte division, the erstwhile normal function of which is the reduction of the diploid number to the haploid in the zygotenic groups; and (4) the mechanism of sex-determination. The cytology of Hymenoptera, therefore, has been presenting tricky problems, the study and understanding of which have proved both fascinating and elusive.

In the above view the author has carried out comprehensive cytological studies of seven representative hymenopterans, involving the highly evolved honey-bee and the primitive saw-fly (Kumbkarni 1961). The present work is to contribute to some of these problems in still another hymenopteran in the context of what has been earlier observed by the author in the closely allied *Apis indica* (Sharma *et al.* 1962) and *A. dorsata* (Kumbkarni 1964), and other Hymenoptera.

Materials and technique

X. fenestata, the common carpenter-bee of Northern India, is one of the rare exceptions in Hymenoptera in which the testes are quite well developed and functional even in the adults. Only for the spermatogonial divisions testes from the just eclosed specimens had been fixed. For oögonial mitotic counts ovaries of freshly emerged females were studied. For ascertaining the somatic numbers of both sexes the dividing larval cerebral ganglionic cells have been studied. For the chromosomal study the best results were achieved from Carnoy fixed iron/haematoxylin preparations. In addition squashes of acetyl alcohol fixed/Feulgen stained and acetyl alcohol fixed/acetocarmine stained material were prepared for determining the spermatogonial, oögonial and somatic complements. Feulgen's nucleal reaction was studied for the 'lagging' chromosomes of male meiosis II.

Living cells were studied with Zeiss stand "W" microscope fitted with Achromatic 1.25 phase-contrast condenser, PH 1.25/100 phase contrast objective with a phase plate having 65 P.C. absorption and K8X Ocular. Wherever necessary photomicrographs were taken with a "Contax" and micro-attachment camera under blue green light (Zeiss filter).

Observations

Haplo-diploid chromosomal relationship: The gonial polar view counts have shown 16 chromosomes in the male (Fig. 1) and 32 in the female (Fig.

13). The haplo-diploidy of this species is further evident by the somatic determinations of 16 and 32 chromosomes in the dividing cells of the cerebral ganglia of the male and female larvae respectively (Figs. 15, 14).

Cytology of spermatogenesis: Abortive meiosis I.—As in *Apis dorsata* the prophase activity is though not distinct, the absence of any synapsis can be fairly well ascertained (Figs. 2, 19, 20). The nuclear area enlarges and single chromosomal threads shorten, thicken and condense to get themselves transformed into 16 metaphase chromosomes (Figs. 2-4 19, 20). The chromosomes are either spherical or thick short rods. Some of them have been observed to be hooked (Fig. 4). The nucleus is stretched pole-wise to

Figs. 1-15. 1, spermatogonial metaphase polar-view revealing 16 chromosomes. 2-5. *Abortive male meiosis I.* 2, prophase. 3, nucleus has elongated pole-wise. Haploid number of 16 chromosomes have been condensed and intra-nuclear spindle fibres have started appearing. 4, intra-nuclear metaphase I polar-view revealing 16 chromosomes. Some of the chromosomes are 'hooked' at one end. 5, intra-nuclear spindle is fully formed and the chromosomes have arranged themselves on its equator. 11, after the disruption of metaphase the chromosomes exhibit certain haphazard movements in small masses. 6, the chromosomes have clumped into a single mass and the intra-nuclear spindle has been lost. The nucleus has been reduced much in size and its membrane becomes wavy. 7-10. *Male meiosis II.* 7, nuclear membrane has disappeared. 8, metaphase II polar-view showing the same haploid number of 16 chromosomes. 9-11, unequal cytoplasmic cleavage results in the production of a diminutive degenerate body and one large functional spermatid. 12, late anaphase II. One chromosome from each group lags behind the others for some time. 13-15. *Oogonial and somatic counts.* 13, oogonial metaphase polar-view giving a diploid count of 32 chromosomes. 14-15, somatic counts of 32 and 16 from the dividing larval cerebral ganglionic cells of the female and male respectively.

acquire a spindle shaped contour (Figs. 3, 5). Within the nucleus a typical bipolar spindle appears and the chromosomes form a compact metaphase plate (Figs. 3, 5). A haphazard non-functional extra-nuclear spindle, so clearly present in *Apis dorsata*, is not discernible in the fixed preparations of this species. However, its presence can easily be detected in the living cells studied under the phase-contrast microscope (Figs. 16, 17). The nuclear membrane, at this stage, is very much thick and sudanophil, and thus rich in lipid content.

The chromosomal activity does not progress beyond this stage. The equatorial arrangement of the chromosomes is disturbed. They get disengaged from the spindle-attachments, clump into small masses, and display haphazard movements along the spindle fibres, quite independent of the latter (Fig. 11). Soon after there is a complete coalescence of the chromosomes into a single mass and the intra-nuclear spindle disintegrates (Figs. 6, 7, 21, 22). The hitherto intact membrane of the now much attenuated nucleus becomes wavy and finally disappears with the result that clumped chromosomal mass comes to lie right in the cytoplasm (Figs. 6, 7). That brings to an end the attempted first meiotic division.

A major deviation from *Apis dorsata* is the non-production of any cytoplasmic bud during the abortive meiosis in the present species, although both of them are related members of the same family, Apidae. The above observation is based upon the study of both the fixed and the living cells.

Meiosis II. Since there has not been even the slightest loss of the cytoplasm in the form of a cytoplasmic bud being pinched off, the size of the cell entering meiosis II remains the same. Without the intervention of any prophase II, 16 discrete chromosomes with well defined outlines reappear out of the chromosomal mass of meiosis I (Figs. 7, 8). Some of the chromosomes appear 'hooked'. A fresh bi-polar spindle is formed, on the equator of which the chromosomes make a metaphase plate. The ensuing anaphase and telophase stages are quite regular, and typical of the mitotic division (Figs. 23-26). However, as in *Apis dorsata*, the cytoplasmic cleavage accompanying the nuclear division is highly unequal with the result that one of the two daughter cells is a small spermatid body, otherwise having a complete complement of the nucleus, mitochondria and, the lipid material—the 'Golgi' elements or dictyosomes (Figs. 9, 10, 26-29). This passes through only the initial stages of the mitochondrial nebenkern, but soon it degenerates. Only the larger sister cell, the main spermatid, undergoes successful spermateleosis.

In quite a few cases it has been observed that a small part of the chromatin material of the would-be spermatid body nucleus is left behind during late anaphase (Fig. 28). Another interesting feature of rather more usual occurrence is that during very late anaphase II, one of the chromosomes from each group seems to lag behind the others. This chromosome

is larger than the rest and is definitely Feulgen positive like the others (Fig. 12). However, it is certain that it is later on included in the corresponding nuclear complement.

Figs. 16-22. *Male meiosis I*: 16, pre-metaphase I stages of male meiosis I, showing the intra-nuclear formation of chromosomes and the elongation of the nucleus (Living study). 17, the nucleus is elongated and has acquired a spindle-shaped contour. The nuclear membrane is very much thickened. The chromosomes have formed a metaphase plate (upper cell) on the equator of an intra-nuclear spindle. Extra-nuclear fibres, though poorly developed, are also discernible (Living study). 18, different stages of degeneration of male meiosis I subsequent to the metaphase. The chromosomes are displaying haphazard movements. The nucleus is getting shortened and its membrane, prior to ultimate dissolution, gets wavy (Living study). 19-20, prophase stages of abortive male meiosis I to show the unpaired nature of the chromosomal threads. 21-22, inhibition of the chromosomal activity at metaphase and subsequent clumping into a single mass. 23-29, *male meiosis II*:—Note the unequal cytoplasmic cleavage. There is sometimes left behind a little chromatin from the proximal telophase mass, that would constitute the spermatid body nucleus (28).

Discussion

Haplodiploidy and chromosomal constitution: Among the bees other than *Apis*, earliest cytological work on record is a partial study of only the male germ cells in *Xylocopa violacea* (Granata 1909, 1913). Sixteen large hooked chromosomes are present in the spermatogonia and the primary spermatocytes: the same number persists in the spermatid. Meiosis I is abortive. However, in the absence of any chromosomal data for the female and the somatic cells of both the sexes, Granata's observations only *inter alia* suggest the haploidy of the male because of the continued presence of 16 chromosomes throughout the germinal track of the male; the corresponding diploidy of the female is left unexplored. By demonstrating the presence of a single genome of 16 and a double genome of 32 chromosomes in the corresponding germ cells and the somatic cells (dividing larval cerebral ganglionic cells) of the male and female respectively, the present investigations constitute the substantial evidence for the haploid male/diploid female relationship in yet another hymenopteran, *X. fenestrata*.

Two other bees, *Osmia cornuta* (Ambraster 1913) and *Euplausia* (cf. *Xylocopa*) *violacea* (as reported by Kerr and Laidlaw 1956) resemble *Xylocopa* and *Apis* species in having the same haploid male/diploid female chromosomal relationship of 16/32. However it is interesting to remark that in five species of an allied genus *Mellipona*, Kerr (1948) has described 9 and 18 as the haploid and diploid numbers respectively. The findings of Whelden (1954) on the bumble-bee, *Bombus firvidus*, suggesting 12 as the chromosome number both for the male and female, are considered untenable in the obvious absence of any endopolyploidy and more so in the context of the prevalence of haplo-diploidy in the Hymenoptera as a whole.

Cytology of spermatogenesis: Abortive meiosis I. Erstwhile reductional meiosis I is either abortive or absent in the Hymenoptera for the reason that the primary spermatocytes have only a single set of chromosomes. The abortion of meiosis I involves the stopping short of the chromosomal activity at either metaphase where an organised spindle (intra-nuclear) is present or immediately after the 'condensation' of the chromosomes when there is no spindle formation. Almost synchronous with this inhibition of division activity a non-nucleated cytoplasmic bud, representing the vestigial cytokinesis, is pinched off in some of the cases (Kumbkarni 1961, Sharma *et al.* 1960, *etc.*). Meiosis I in *X. fenestrata* is in a sense more abortive than in *Apis* and some other Hymenoptera because the cytoplasmic bud (*opt. cit.*), usually considered a relic of the erstwhile secondary spermatocyte, is quite significantly not produced. In the apidae itself *X. fenestrata* resembles *Osmia cornuta*, tribe Megachilini (Ambruster 1913) and *Euplausia violacea*, tribe Euglossini (reported by Kerr and Laidlaw 1956) in the non-production of a cytoplasmic bud. It seems that the bud is always given off in those

bees which occupy a position close to *Apis*, tribe Apini, in the tree of phylogenetic relationship of the bees as enunciated by Michner (1944). Kerr and Laidlaw (1956), relying upon Granata's claims on the production of cytoplasmic bud in *X. violacea*, comment that according to Michner Xylocopini is removed more from Apini than Euglossini, but it may be possible that Xylocopini is infact closer to Apini than what Michner shows. If some pylogenetic importance is to be ascribed to those phenomena, the present work by showing the absence of any bud formation in Xylocopini (confirmed by the study of living cells under the phase-contrast microscope) re-affirms the systematic portion of Xylocopini away from Apini as originally suggested by Michner.

However it may be pointed out that in addition to the above mentioned apids, the expulsion of a cytoplasmic bud has also not been observed in some other Hymenoptera of divergent systematic positions, e.g., *Pteronidea* (Sanderson 1932), *Diprion* (Smith 1941), *Mellitobia* (Schmieder 1938), *Camponotus compressus* and *Polistes hebraeus* (Kumbkarni 1961).

Keeping in view that apart from the presence or otherwise of the cytoplasmic bud formation there are important differences in the abortion of meiosis in the various evolutionary grades and sometimes closely allied species, it is imperative to concede that such a regulation of meiosis has probably a multiple origin in the evolution of male haploidy in Hymenoptera.

Though a regular prophase is not discernible yet it has been possible to ascertain that at no stage the chromosomal threads undergo any synapsis as claimed by Sanderson (1932) and Greenshields (1936) for the saw-fly, *Pteronidea ribesii* and by Dodds (1938) for the gall-wasp, *Neuroterus baccarum*. The tetrad formation as such is not possible because of the presence of only one set of maternal chromosomes in the primary spermatocytes. Therefore the traditional significance attached to the abortive meiosis I that it checks a further reduction of the already haploid number of chromosomes in the male is not a proper interpretation of the facts. Instead it may be suggested that the male meiosis I, having lost all its functional significance in the course of evolution of male haploidy, is represented in the existing Hymenoptera by just a degenerate vestige of the erstwhile normal meiosis I of the now extinct ancestral zygogenetic diploid male Hymenoptera.

Meiosis II. As in other Hymenoptera the metaphase II chromosomes reappear directly from the chromosomal mass of the abortive meiosis I without the intercalation of even a semblance of prophase activity as witnessed in *Apis dorsata* (Kumbkarni 1964).

The cytoplasmic cleavage being highly unequal, one large spermatid and a diminutive spermatid body are formed. Just like other apids the spermatid body degenerates and only one sperm is produced. As in *A. indica* (Sharma *et al.* 1962) and *A. dorsata* (Kumbkarni 1964) there is no evidence that the lagging chromosome from each anaphase group is not ultimately

included in the nuclear reconstruction of the respective spermatid cells. Therefore the temporarily lagging chromosome of the present observations cannot be compared to the 'X-chromosome' of *A. mellifica* as suggested by Manning (1950, 1952). The author, however, is inclined to believe that the 'multiple sex allele' theory of sex-determination, originally postulated for *Habrobracon* (Whiting 1945) and *A. mellifica* (Rothenbuhler 1957), is equally valid for other Hymenoptera also.

Summary

X. fenestrata has been found to be arrhenotokously parthenogenetic, and the haploid male/diploid female chromosomal relationship is 16/32. The male meiosis I is abortive but a cytoplasmic bud, usually considered as the vestigial secondary spermatocyte, is not produced. The second spermatocyte division is also anomalous as only one functional spermatid is formed.

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