

ULTRASTRUCTURE OF SINGLE AND MULTIPLE DIPLOID HONEYBEE SPERMATOZOA¹

J. WOYKE

Bee Division, Agricultural University, 02-766 Warszawa 13, Nowoursynowska 166, Poland

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Summary

Diploid drone honeybees were reared by Woyke's method and the ultrastructure of diploid and haploid spermatozoa was investigated. The ultrastructure of diploid spermatozoa is very similar to that of the haploid ones, but the diploids are larger. The length of the acrosome and the nucleus of a diploid spermatozoon are 151% and 139% respectively of those for a haploid one. The diameter of the tail of a diploid spermatozoon is 115% of that of a haploid one. A single axoneme with an arrangement of $9 + 9 + 2$ fibrils is present in a diploid spermatozoon. The direction of helical grooves is the same on both mitochondrial derivatives in diploid spermatozoa, whereas it is opposite on the two derivatives in haploid spermatozoa. The volume of a diploid spermatozoon is about twice that of a haploid one.

Among ordinary diploid spermatozoa were also found double ones, containing two axonemes and four mitochondrial derivatives in their tails, and triple ones, containing three axonemes and six mitochondrial derivatives. The diameters of the tails of double and triple spermatozoa are 165% and 195% respectively of the diameter of the tail of a single diploid spermatozoon, but the diameter of each axoneme is the same whether the spermatozoon is single or multiple. In multiple spermatozoa the normal association of a pair of mitochondrial derivatives with each axoneme is not seen. It is concluded that multiple spermatozoa are not formed by fusion of mature spermatozoa, but probably arise by premature spermiogenesis in spermatoids that have not yet separated.

Introduction

Normal honeybee drones are haploids, because they develop from unfertilized eggs, and, consequently, no reduction in the number of chromosomes can occur during their spermatogenesis (Meves, 1907). Woyke (1963) reported that diploid drones may develop from fertilized eggs and reared them to the adult stage (Woyke, 1969). Woyke and Skowronek (1974) found that reduction in the number of chromosomes during spermatogenesis does not occur even with diploid drones. The spermatozoa are diploid and contain twice as much DNA as the haploid ones (Woyke, 1975). The lengths of a whole diploid spermatozoon and its head are 130% and 154% of those of a haploid one (Woyke, 1983).

Spermiogenesis in haploid drones was described by Orska (1938) and Hoage and Kessel (1968), but they did not investigate diploid drones. The ultrastructure of haploid spermatozoa was described by Rothschild (1955), Hoage and Kessel (1968), Cruz-Höfling et al. (1970) and Lensky et al. (1979). Again, diploid drones were not investigated so the effects of diploidism on the structure of spermatozoa are not known. Phillips (1970*b*) and Baccetti (1972) have extensively reviewed papers on insect sperm cells.

Materials and Methods

Diploid drones were reared by Woyke's method (1969). According to our observations their spermatozoa rarely pass into the seminal vesicles, and mostly remain in the testes. Spermatozoa were therefore collected from the testes of emerging drones, because it was feared that spermatozoa in older drones might have degenerated. Spermatozoa from haploid drones were collected from ones of the same age. The testes were dissected in physiological solution, (1.5% NaCl) and subjected to one of two procedures. For whole mounts, the tubules were cut in pieces in a drop of water on a microscope slide. The released spermatozoa were transferred to a membrane-coated grid and left to dry. Then they were dusted with carbon, generated by an electric arc. Spermatozoa prepared in this way were only used to study the morphology of their heads, and they were always compared with fixed sperms in sections. For sectioning, the testes were fixed in phosphate buffered 5% glutaraldehyde (pH 6.9), followed

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by post-fixation in 1% OsO₄. After dehydration in ethanol, the testes were embedded in Epon-812 and sectioned. The sections were stained with lead citrate and uranyl acetate and examined with a Japanese (Jeol) electron microscope model JEM-100C.

Results

The head of the diploid spermatozoon

The head is composed of an acrosome and a nucleus (Fig. 1).

The acrosome

The acrosome is flattened and lanceolate (Fig. 1, Fig. 2), 5.3 µm long and 0.65 µ wide. Its length is 150% of that of the haploid spermatozoon (3.5 µm). The tip, which acts as a perforatorium, is of low optical density. Inside the acrosome there is an electron dense filament, which does not reach the perforatorium. It also is flat, 0.40 µm wide and 0.07 µm thick. Cross sections show two parallel cavities 0.06 µm diameter traversing the length of the acrosomal filament (Fig. 2A). Longitudinal or oblique sections show them as two tubules (Fig. 2B). The posterior end of the acrosomal filament penetrates the nucleus.

The nucleus

The nucleus also is flattened, and is of high electron density (Fig. 2A). No internal structure is apparent. Anteriorly it ends obliquely (Fig. 3A), and has a lateral groove penetrating further into it (Fig. 3B). The posterior end of the acrosomal filament enters the groove and dives into the nucleus beside it. The posterior end of the nucleus is cone-shaped (Fig. 3C) and its tip has a finger-like projection surrounded by fibrils. Grooves or projections are seen at the cone where the mitochondrial derivatives fit. Excluding the finger-like projection the nucleus is 7.36 µm long, 0.65 µm wide and 0.275 µm thick. Its length and width are 139% (5.3 µm) and 130% (0.5 µm) respectively of those of a haploid spermatozoon.

The tail

The tail is cylindrical with a snake-like posterior tip. Its diameter is 0.75 µm, which is 115% of that of a haploid spermatozoon (0.65 µm). Inside are an axoneme (flagellum), two mitochondrial derivatives and two triangular rods (Fig. 4, Fig. 5, Fig. 6).

The axoneme

The axoneme is cylindrical and located eccentrically in the tail (Fig. 4). This side of the spermatozoon is denominated ventral. The axoneme begins at the cone-shaped region of the nucleus and has a snake-like ending before the tip of the tail of the spermatozoon. The diameter of the axoneme is about 0.235 µm, which is similar to that of the haploid spermatozoon. Inside there are 9 peripheral accessory tubular fibrils, whose diameter is 0.02 µm, each with an electron-dense rod in its centre (Fig. 7).

Further in, 2 double fibrils are visible. One is a little larger than the other. The smaller one is provided with two lateral arms. The doublets are not located directly beneath the outer fibrils, but are shifted tangentially in one direction. A substance more dense for the electrons is present between each outer fibril and its nearest shifted doublet. As a result a catherine-wheel-like structure is formed. Each double fibril is connected by a radius with a crescentic structure located more centrally (Fig. 4, Fig. 7). The convex face is turned outwards. Two dense fibrils, 0.02 µm in diameter, are present in the centrum of the axoneme. They are separated by a 0.01-µm gap and interconnected by two circiform linkages. The general scheme of the fibrils in the axoneme is thus 9 + 9 + 2.

The mitochondrial derivatives

The mitochondrial derivatives are two elongated structures located eccentrically in the tail. One of them is larger (Figs 4–6) and longer than the other. They begin in the region of the cone of the nucleus (Fig. 7B), and continue throughout most of the tail, but they do not reach its tip and end anteriorly to the tip of the axoneme. They appear in transverse sections as semicircles, triangles, ellipses, or similar forms (Figs 4–6). The derivatives are electron-dense structures composed of amorphous material. In the posterior portion a spongy material can be

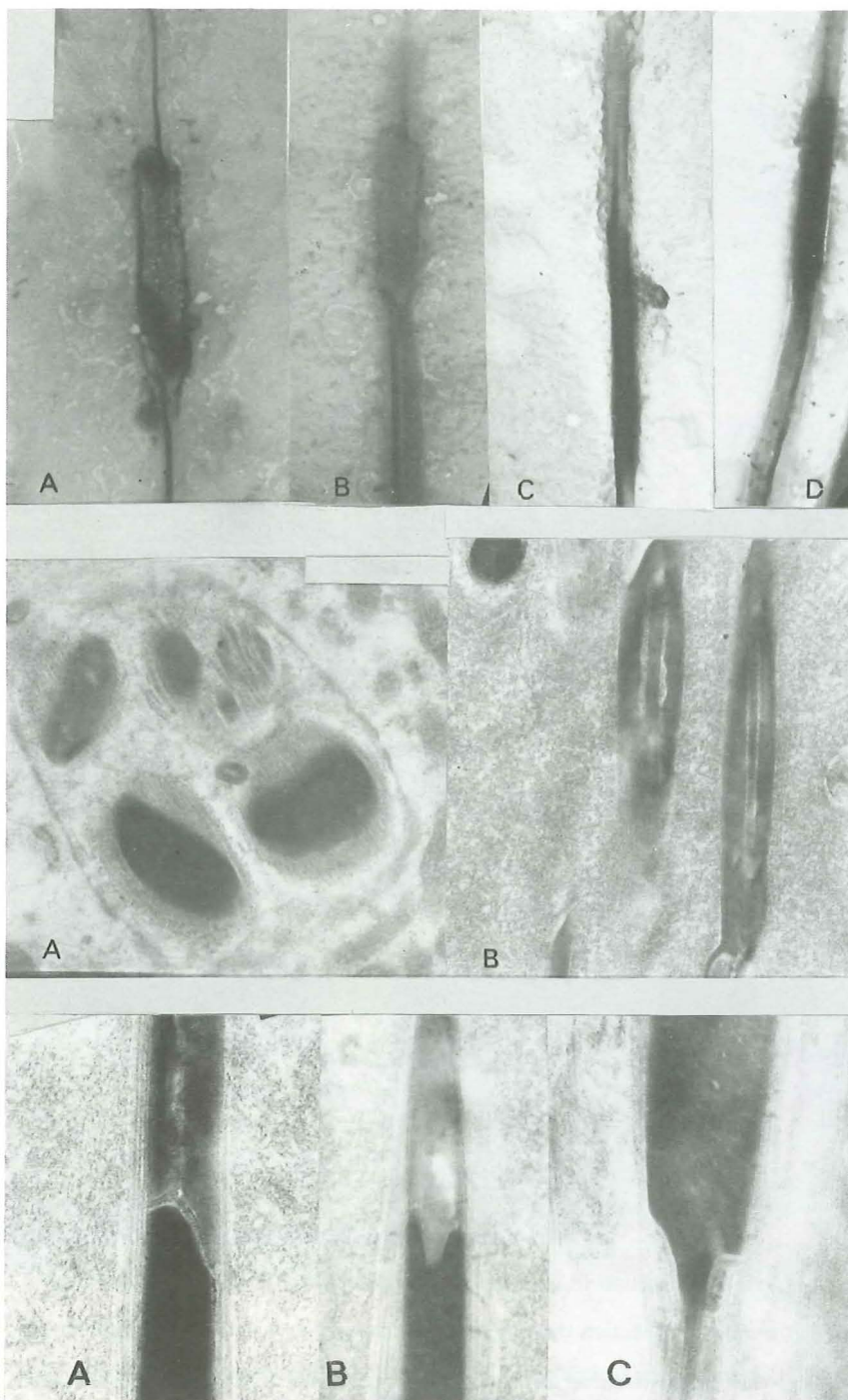


FIG. 1. (*top*). Heads of spermatozoa $\times 5000$. A. Single diploid, broadside view, B. Haploid, broadside view, C. Single diploid, edgewise view, D. Haploid, edgewise view.

FIG. 2 (*centre*). Single diploid spermatozoa in a cyst. A. Cross section through: acrosome (*above left*), tail (*above right*) and nuclei (*below both*) $\times 26\ 000$, B. Oblique section through acrosome $\times 16\ 000$.

FIG. 3. (*bottom*). Nuclei of single diploid spermatozoa $\times 26\ 000$. A. and B. Anterior tip, C. Posterior tip.

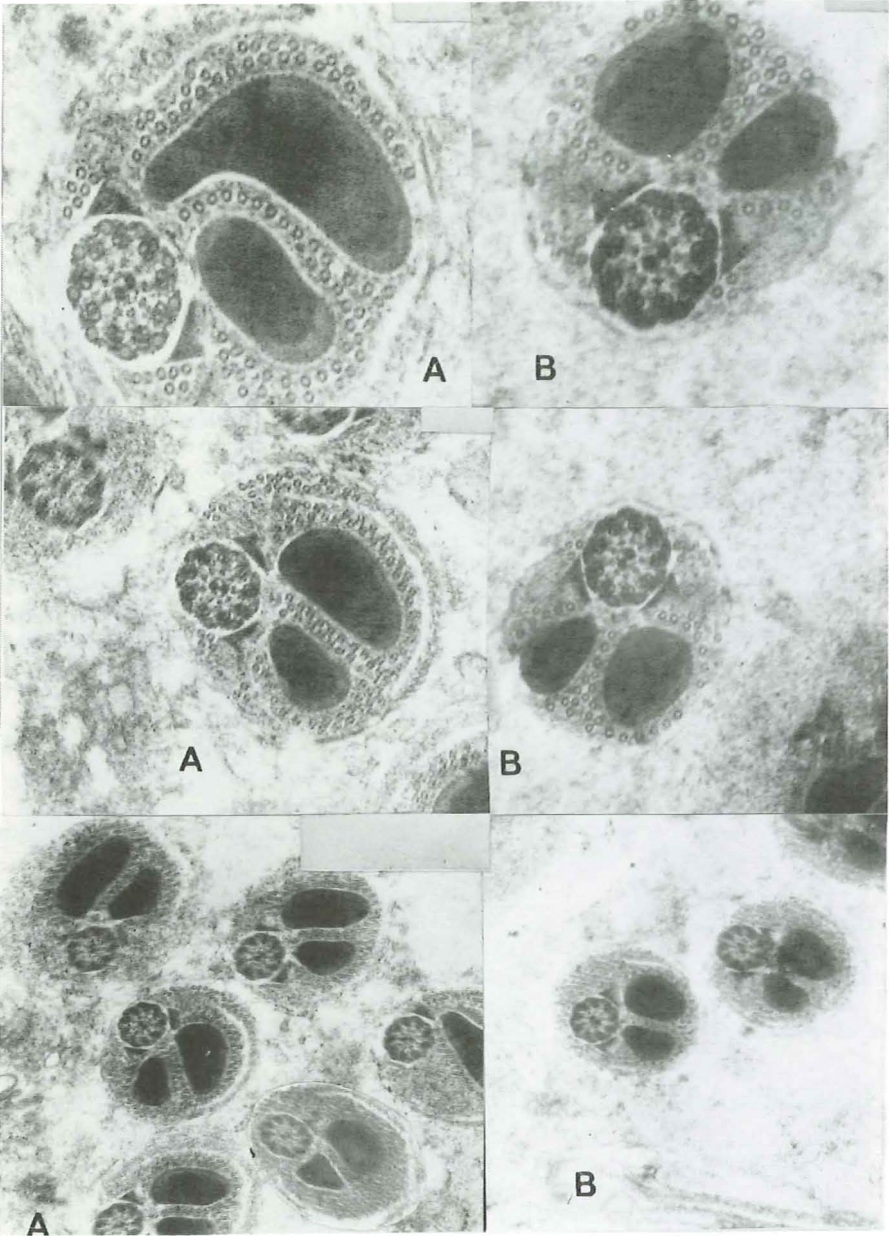


FIG. 4. (*top*). Cross section through tail of spermatozoon $\times 66\ 000$, A. Single diploid, B. Haploid.

FIG. 5. (*centre*). Cross section through tail of spermatozoon $\times 50\ 000$, A. Single diploid, B. Haploid.

FIG. 6. (*bottom*). Cross sections through tails of spermatozoa $\times 26\ 000$, A. Single diploid, B. Haploid.

seen in the central part. The peripheral region of the derivatives is covered by less dense material. This mass does not entirely surround the derivatives, but is present mainly on their outer sides, and is absent on the surfaces where the derivatives face each other. Helical fissures in this less dense material are disposed obliquely at an angle of 60° to the longitudinal axis of the

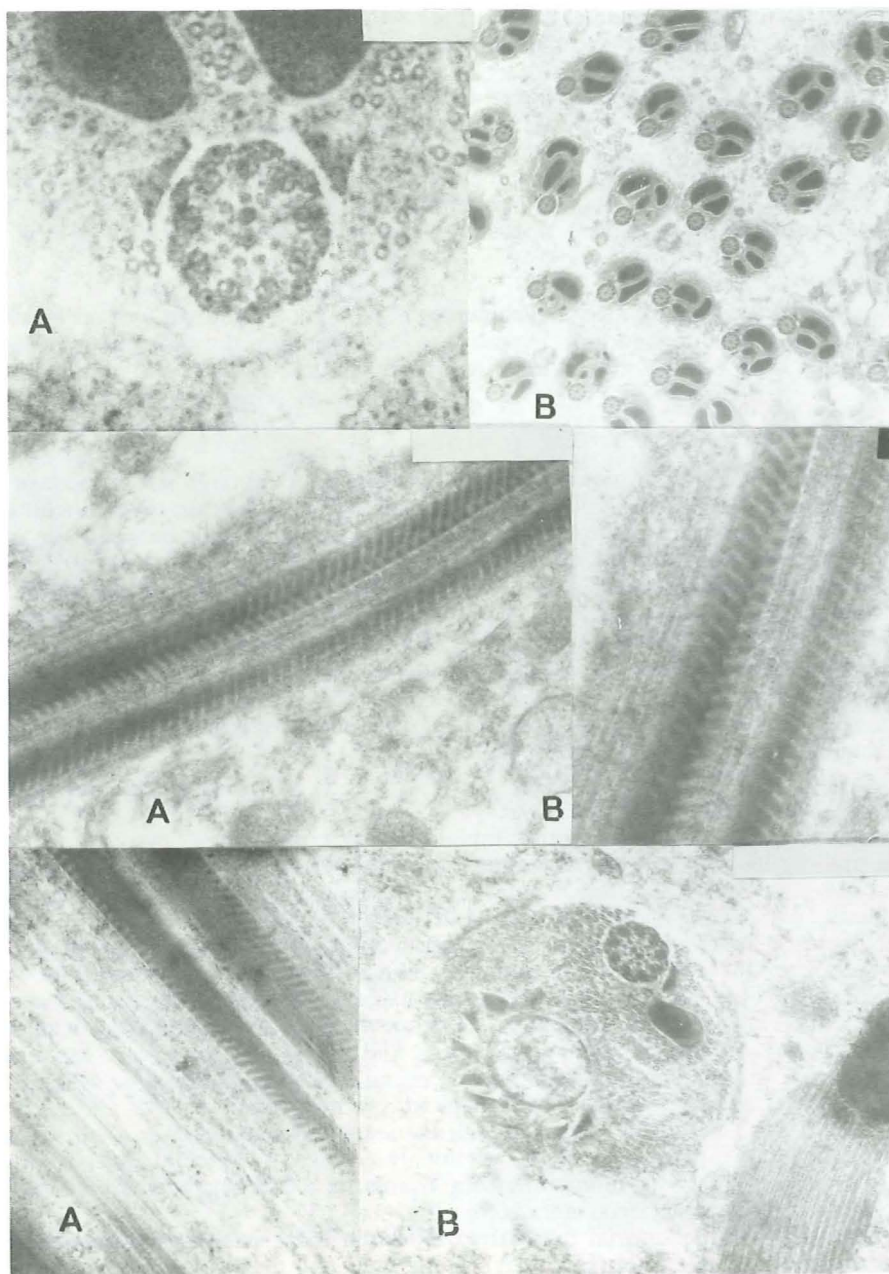


FIG. 7. (*top*). Cross section through: A. Axoneme of single diploid spermatozoon $\times 100\ 000$, B. Intermediate zone between nucleus and tail $\times 10\ 000$.

FIG. 8. (*centre*). A. and B. Mitochondrial derivatives of single diploid spermatozoa $\times 33\ 000$.

FIG. 9. (*bottom*). A. Oblique section through mitochondrial derivatives of single diploid spermatozoon $\times 33\ 000$, B. Cross section through tail of irregular diploid spermatozoon $\times 33\ 000$.

spermatozoon, forming the so-called cristae (Figs 8–9A). The grooves are $0.03 \mu\text{m}$ deep and are $0.045 \mu\text{m}$ apart. Their depth and spacing are similar to those found in this investigation for haploid spermatozoa.

Cruz-Höfling et al. (1970) and Lensky et al. (1979) say that in a haploid spermatozoon the helices are in opposite directions in the two derivatives. In diploid spermatozoa, however, a line can be found, within a single derivative, from which the grooves start in opposite directions (Fig. 8). As a result, in longitudinal or oblique sections, grooves can be seen that run over both derivatives in the same or opposite directions. Mostly, however, fissures running in the same directions are visible in both mitochondrial derivatives (Fig. 9A).

Triangular rods

Two triangular rods lie parallel to the axoneme (Figs 4–7A). They are located between the axoneme and the two mitochondrial derivatives. The bases face the axoneme and the apices point outwards. The base of the triangle measures about $0.125 \mu\text{m}$ and the height about $0.055 \mu\text{m}$.

Microtubuli

Microtubuli are present in spermatozoa of drones (Figs. 4–7A), though not in those of mature drones (Hoage & Kessel, 1968). The acrosome as well as the nucleus are covered with a sheath of longitudinal microtubuli. In the tail, the microtubuli are present between the different structures as well as in the peripheral zone, but they were never found between the axoneme and the triangular rods.

Irregular diploid spermatozoa

Sometimes vacuoles are present in the tails of diploid spermatozoa. Fig. 9B shows a section through a tail with one axoneme, one mitochondrial derivative and one vacuole. Only one triangular rod is visible between the axoneme and the mitochondrial derivative, but eight or nine of them are present around the vacuole.

Double diploid spermatozoa

Cross sections through tails of some diploid spermatozoa show two axonemes, four mitochondrial derivatives and four triangular rods (Figs. 10–12). Such spermatozoa are called 'double'. The diameter of the tail is $1.235 \mu\text{m}$, which is 164% of that of a single diploid spermatozoon, and 190% of that of a haploid one.

The diameter of the axonemes is $0.235 \mu\text{m}$, which is the same as in single diploid spermatozoa. The disposition of the axonemes inside the tail varies. Sometimes they are located centrally (Fig. 10A). In other cases, one axoneme is in the centre and the other in peripheral zone (Fig. 10B). In still others, both are located peripherally, and can be close together (Fig. 11A), or diametrically opposed (Fig. 11B, Fig. 12A). Inside each axoneme, nine peripheral fibrils, nine inner double, and two central ones are visible. The scheme is thus $9 + 9 + 2$, just as in single diploid and haploid spermatozoa.

Usually two triangular rods lie alongside each axoneme, but sometimes an axoneme has only one rod or none at all. The triangular rods do not always face the mitochondrial derivatives as they do in single spermatozoa. Sometimes one triangular rod is on the opposite side of the axoneme from any mitochondrial derivative (Fig. 11A).

Cross sections of mitochondrial derivatives are often more irregularly shaped in double spermatozoa than in single ones (Fig. 11A). Sometimes two mitochondrial derivatives unite creating one large and two smaller ones (Fig. 12A). The disposition of the four mitochondrial derivatives inside the tail also varies considerably. Regular association of two mitochondrial derivatives with each axoneme can be seen only exceptionally. Fig. 12B shows a section where there is a pair of derivatives associated with one axoneme, but one derivative of the other pair is under the influence of one axoneme and the other under the influence of the other axoneme. More often, however, there is no association of a pair of derivatives with a particular axoneme (Figs 10–11). Inside some derivatives a spongy substance is seen, although more peripherally the normal amorphous, electron-dense material is present and part of the periphery is covered with the usual layer of lower electron-density material. This material is disposed more irregularly in double spermatozoa than in single ones. Sometimes it covers opposite sides of a

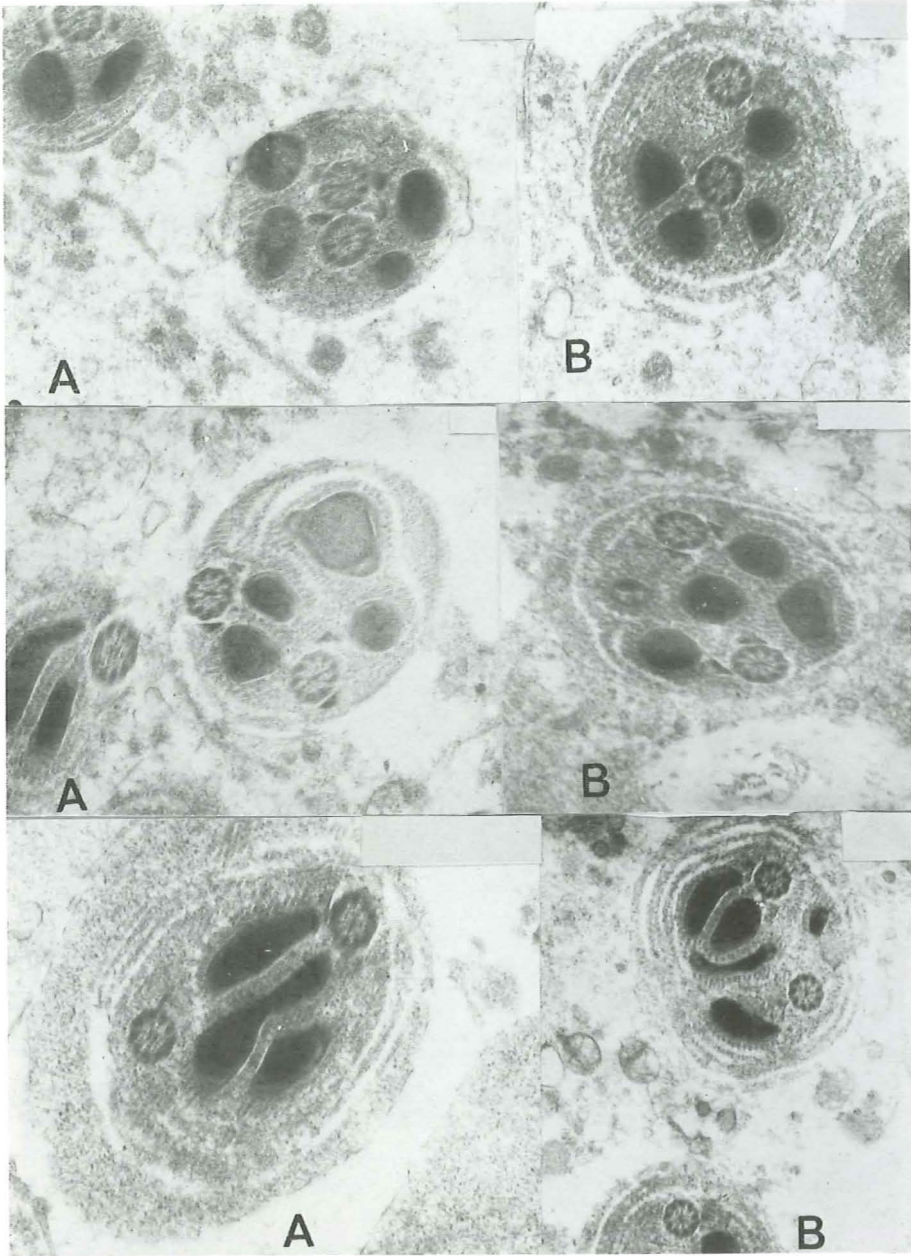


FIG. 10. (*top*). Cross sections through tails of double diploid spermatozoa $\times 26\ 000$, A. Both axonemes in the centre, B. One axoneme in the centre and the other on the periphery.

FIG. 11. (*centre*). Cross sections through tails of double diploid spermatozoa $\times 26\ 000$, A. Both axonemes on the periphery, B. Axonemes diametrically opposed.

FIG. 12. (*bottom*). Cross sections through tails of double diploid spermatozoa, A. Two mitochondrial derivatives fused $\times 26\ 000$, B. One pair of mitochondrial derivatives associated with one axoneme $\times 20\ 000$.

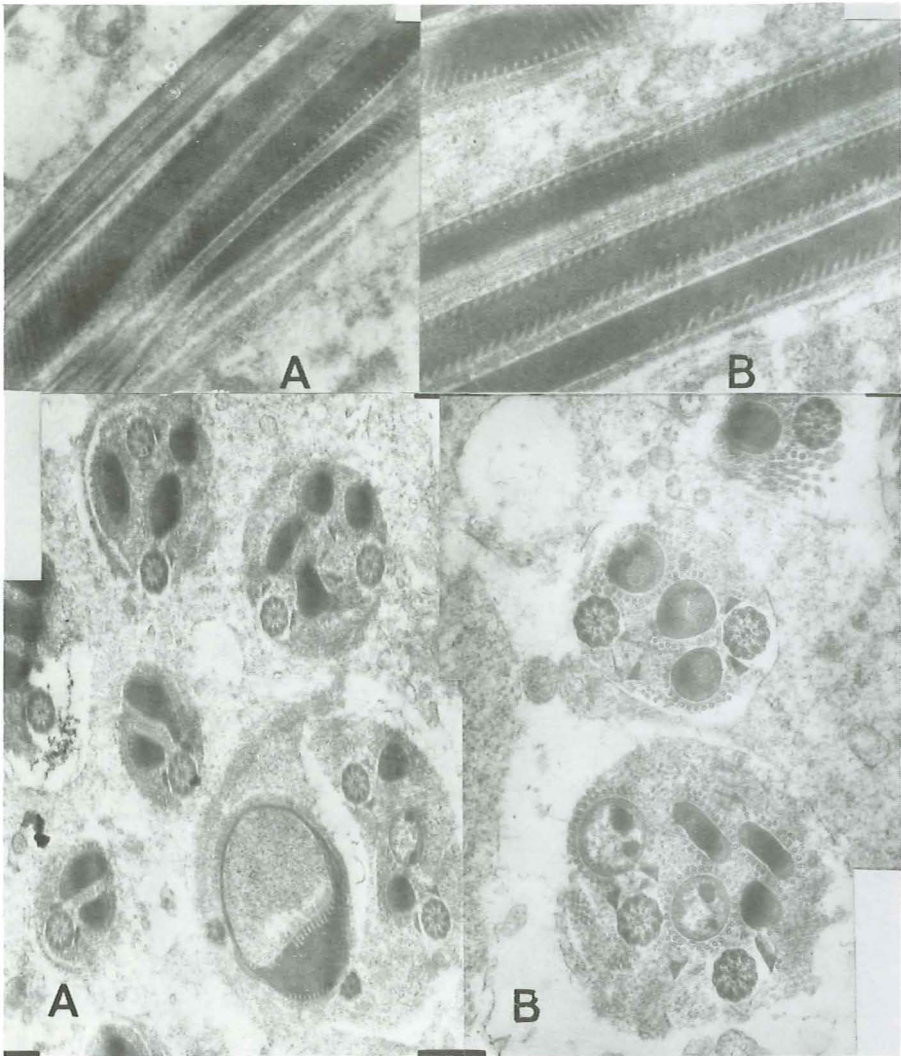


FIG. 13. (*top*). Identical directions of grooves on three mitochondrial derivatives of diploid multiple spermatozoa, A. Oblique section $\times 26\ 000$, B. Longitudinal section $\times 33\ 000$.

FIG. 14. (*bottom*). Cross sections through tails of irregular double diploid spermatozoa, A. Enlarged mitochondrial derivatives $\times 20\ 000$, B. Vacuoles inside the tail $\times 26\ 000$.

derivative (Fig. 11A). Helical grooves are present in this mass. Oblique sections (Fig. 13A), or longitudinal ones (Fig. 13B), show that the directions of the helices are identical on all three mitochondrial derivatives.

Various irregularities may be found in the tails of some double spermatozoa. One mitochondrial derivative may be enormously enlarged and filled with spongy material inside (Fig. 14A). Vacuoles with electron-dense inclusions may be seen in some others (Fig. 14B). Probably the vacuoles are transformed mitochondria, but no proof of this is presented.

Triple diploid spermatozoa

Transverse or oblique sections through tails of some spermatozoa show three axonemes and six mitochondrial derivatives (Fig. 15, Fig. 16A). Such spermatozoa are called 'triple'. No exactly

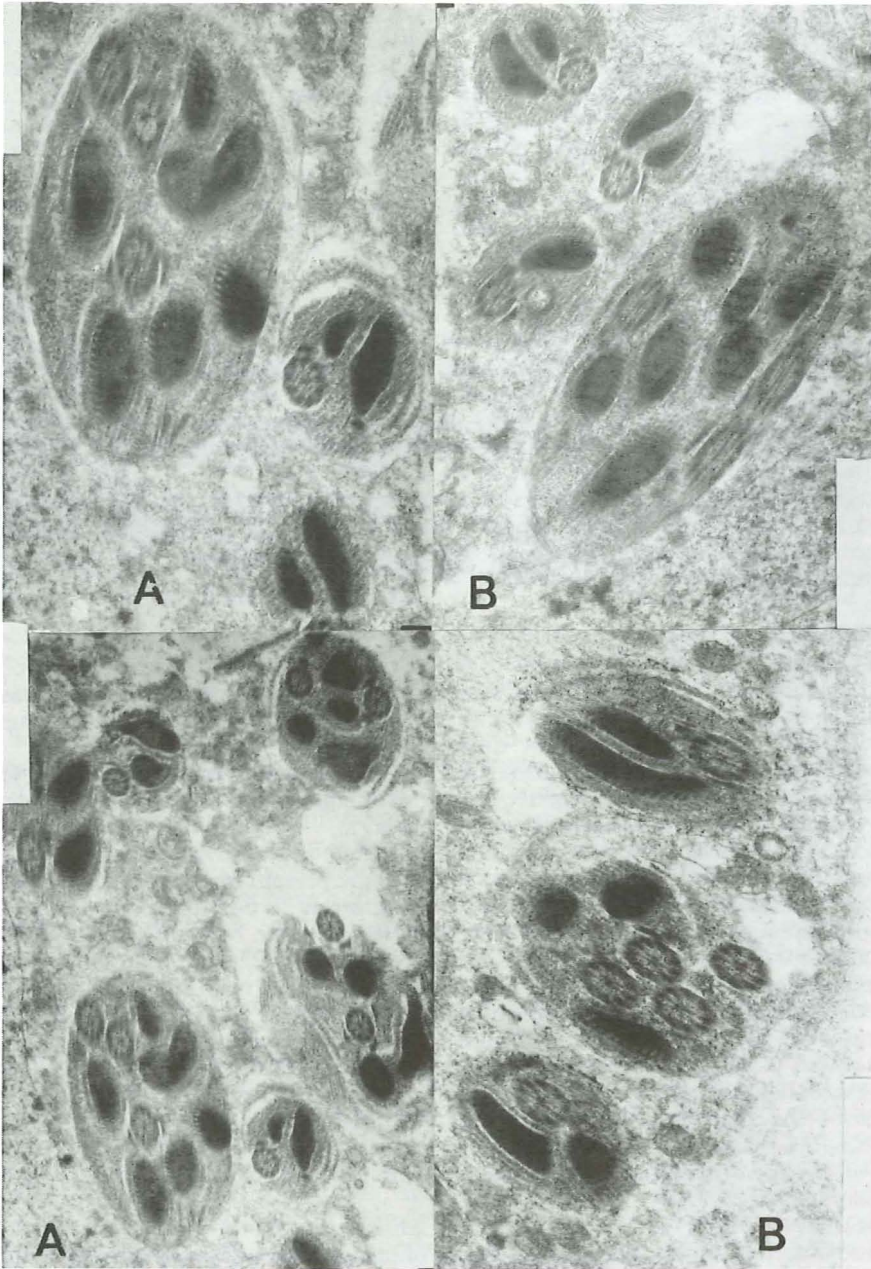


FIG. 15. (*top*). Sections through tails of single and triple diploid spermatozoa, A. One axoneme in the centre and two on the periphery $\times 26\ 000$, B. All three axonemes on the periphery $\times 20\ 000$.

FIG. 16. (*bottom*). A. Single, double and triple diploid spermatozoa $\times 16\ 000$, B. Diploid spermatozoon with four axonemes (quadruple) $\times 26\ 000$.

transverse sections were found, and it is difficult to decide from the oblique ones whether the tails are round or elliptical. The shortest diameter through obliquely cut tails is about $1.435\ \mu\text{m}$ which is 190% of that for single diploid spermatozoa and 220% of that for haploid ones.

The diameter of each axoneme in triple spermatozoa is $0.235\ \mu\text{m}$, which is the same as it is in single diploid spermatozoa. The disposition of axonemes in the tail varies. One axoneme may be located in the centre and two others in the periphery (Fig. 15A), or all three may be in the periphery (Fig. 15B). The scheme of the internal structure is $9 + 9 + 2$ fibrils, just as in haploid or single diploid spermatozoa.

In oblique sections, mitochondrial derivatives of triple spermatozoa are mostly elliptical. Association of a pair of them with each axoneme cannot be seen. The internal structure is similar to that found in single or double spermatozoa. Direction of helical grooves is the same in all six of the mitochondrial derivatives visible on Fig. 15 and Fig. 16A.

Quadruple diploid spermatozoa

Fig. 16B shows a section through a tail with four axonemes and three mitochondrial derivatives. The diameter of the tail is about $1.0\ \mu\text{m}$, which is larger than that of a single diploid spermatozoon, but smaller than those of double and triple spermatozoa. Thus the section was probably made through the tail of a quadruple spermatozoon near the posterior end where some mitochondrial derivatives had already terminated, although the possibility cannot be excluded that this is simply the tail of a spermatozoon with four axonemes but only three mitochondrial derivatives.

Although diploid spermatozoa were quite commonly found to be multiple in the way described above, multiple haploid spermatozoa seem to be rare. The only examples found were from one haploid drone whose spermatozoa each had two axonemes but only two mitochondrial derivatives (Fig. 17B).

Discussion

According to Woyke (1983) the lengths of entire diploid spermatozoa ($313\ \mu\text{m}$) are 129% of those of haploid ones ($242\ \mu\text{m}$). Various structures of haploid spermatozoa were measured by Cruz-Höfling et al. (1970) and Lensky et al. (1979). Their measurements mostly agree with those given here for the haploids, or differ only slightly.

Compared with those of spermatozoa treated with Feuglen reaction (Woyke, 1983) the diploid nuclei now described are slightly shorter and haploid nuclei are slightly longer, so the ratio of diploid to haploid lengths is slightly less.

The depth of the helical grooves (cristae) on mitochondrial derivatives found by the above authors was $0.01\ \mu\text{m}$, whereas $0.03\ \mu\text{m}$ was found in the present investigation for both haploid and diploid spermatozoa. The distance between grooves ($0.045\ \mu\text{m}$) now found for both haploid and diploid spermatozoa agrees with the $0.04\ \mu\text{m}$ given by Lensky et al. (1979) but is larger than the $0.025\ \mu\text{m}$ given by Cruz-Höfling et al. (1970). The latter author reports 30° as the angle between the direction of the oblique grooves on the mitochondrial derivatives and the longitudinal axis of the spermatozoon, whereas Lensky et al. (1979) give 60° . The angle now found is 60° for both haploid and diploid spermatozoa. Both the above groups of authors agree that the directions of the helical grooves are opposite in the two mitochondrial derivatives of haploid spermatozoa, but in diploid spermatozoa it now appears that the directions of the grooves are usually the same in both.

Since the linear dimensions of single diploid spermatozoa have been found to be 115–151% of those of haploid spermatozoa, the volume of diploid spermatozoa could be roughly twice that of haploid ones.

The best description of spermiogenesis of haploid spermatozoa is presented by Orska (1938). She describes the retarded development of one mitochondrial derivative, which results in its smaller size. She suspects that this is caused by the haploidy of the drone, and the lack of equal division of the spermatocytes. Woyke and Skowronek (1974) found that during spermatogenesis in drones, polar bodies are created and equal division of spermatocytes does not occur. The smaller size of one mitochondrial derivatives in diploid spermatozoa suggest that the same process of spermiogenesis occurs in diploid drones, where it cannot be caused by haploidy, but might be caused by the abortive division of the spermatocytes. The internal structures of multiple spermatozoa that were observed on oblique or longitudinal sections lay parallel to each other throughout the sections. This demonstrates that the sections show intergrated cells and not locally united spermatozoa. The larger diameter of tails of multiple spermatozoa, and the fact that the diameter of each axoneme in a multiple spermatozoon is

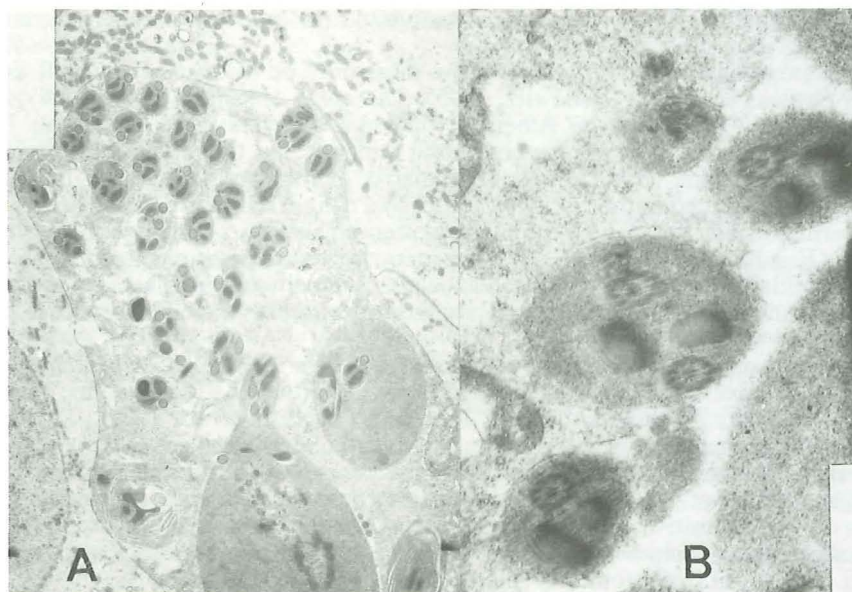


FIG. 17. A. Section through testicular tubule of diploid drone showing some multiple diploid spermatozoa in a mass of plasma $\times 15\ 000$, B. Cross section through tail of haploid spermatozoa with two axonemes $\times 20\ 000$.

equal to that in a single spermatozoon suggests that a multiple spermatozoon does not originate from a single spermatocyte or spermatid in the way that a single spermatozoon does. A double spermatozoon must come from two spermatocytes or spermatids and a triple one from three. However, the irregular disposition of the different internal structures inside the tails and the lack of association of a pair of mitochondrial derivatives with each axoneme suggest that a multiple spermatozoon is not made by fusion of two or more mature single spermatozoa. The process of spermiogenesis must have occurred in a syncytium of unpartitioned spermatocytes or spermatids, or enlarged (giant) spermatocytes and spermatid. Sometimes several spermatozoa can be found in a larger mass of protoplasm, at least in newly emerged drones (Fig. 17A).

Meves (1907), Orska (1938) and Hoage and Kessel (1968) reported that the spermatocytes of haploid drones are interconnected and separation occurs only in the spermatid stage. Fyg (1973) showed experimentally that, in haploid drones ligatured behind the head or removed from their cocoons, enlarged spermatocytes and spermatids occurred; polar bodies were not eliminated and multinuclear spermatids were produced. Spermatozoa with double length heads were encountered. Tarelho (1981a) found giant spermatocytes and polyploid metaphases in haploid drones treated with high and low temperatures respectively. She encountered also binucleate spermatids and spermatozoa in drones treated with low temperature (1981b). Ultrastructure of all those spermatozoa was not investigated. Kerr (1972) found spermatocytes with two nuclei in *Malipona marginata* pupae exposed for 5 days to a low temperature. The present author also has found multinuclear spermatocytes in *Apis mellifera*, though the cause of them was not known (unpublished). Such spermatocytes might develop into multiple haploid spermatozoa. Hoage and Kessel (1968) found two spermatozoa with two flagellae (axonemes), among the many haploid ones investigated by them. The number of mitochondrial derivatives in those spermatozoa was not reported.

Polyploid or multiple structures are also found in spermatozoa of other insects, but it seems that all of them are of different nature from the honeybee ones.

Giant or polipyrene spermatozoa mixed with haploid ones were reported to occur in Cleoptera, Bruchidae (Mulnard, 1951), Carabidae (Bouix, 1963) and Orthoptera (Richards, 1963). It is not clear from the descriptions which of the polipyrene were polyploid and which were multi-nucleate. They arose from giant spermatocytes and spermatids. Probably

endomitosis occurs here. Some of the giants were multinucleate. The ultrastructure of these spermatozoa was not described.

Two spermatozoa may be connected by the head only or by part of the tail, as in the coleopteran *Dytiscus marginalis* (Ballowitz, 1895), *Thermobia domestica* (Bawa, 1964), *Lepisma saccharina* (Werner, 1964), or the American opossum (Biggers & Creed, 1962; Phillips, 1970a). The individuality of the two spermatozoa remains, and the plasma membrane of the two units remain separate (Bawa, 1964). The matrix of the spermatozoon of the silkworm *Bombyx mori* is surrounded in the ejaculatory duct by a cylindrical sleeve. Most sleeves contain only one spermatozoon, but a small number of exceptional sleeves contain two or even three spermatozoa (Friedländer & Gitay, 1972). The spermatozoa are not integrated and association of two mitochondrial derivatives with each axoneme is clearly visible. This is contrary to the lack of association of two mitochondrial derivatives with each axoneme in honeybee multiple spermatozoa.

Two or three axonemes were found in spermatozoa of a sea urchin (Afzelius, 1959), the louse *Pediculus humanus corporis* (Ito, 1966) and the whole group of Rhynchotoids: Psocoptera, Mallophaga, Anoplura, Thysanoptera and Rynchota (Baccetti et al., 1969). The doubling involved only the axonemes. There were only two mitochondrial derivatives there or even only one, whereas in diploid multiple spermatozoa of the honeybee, the numbers of mitochondrial derivatives as well as of axonemes, are multiplied. Spermatozoa with two axonemes were found only in the primitive insect orders and not in the most evolved orders.

In the fungus gnat *Sciara coprophila* the axoneme consists of 70 doublets, instead of the usual 9 + 9 + 2 patters of tubules (Phillips, 1966).

Spermatozoa of three species of hopper (Membracidae) branch into four tails with 2 + 2 or 3 + 3 tubules in the axoneme (Phillips, 1969). Spermatozoa of the termite *Mastotermes darwiniensis* have about 100 flagellae.

Abnormal tails are also associated with absence of one sex chromosome or presence of supernumerary chromosomes. In *Drosophila melanogaster* males that have X/O sex chromosomes instead of the normal set of X/Y, abnormalities occur during development of spermatozoa. Attachment between axonemes and mitochondria is disorganized. Some cases are found where two axonemes appear to be attached to the same mitochondrion whereas other axonemes are not closely associated with any mitochondria. The character is thus similar to what is found in haploid honeybee spermatozoa, but in contrast to the honeybee no fully mature sperms are present in the testes of X/O *Drosophila* males (Kiefer, 1966). On the other hand X/Y/Y *Drosophila* males produce sperms twice as long as those of X/Y males. Thus doubling of sex chromosomes results in larger spermatozoa, as in the honeybee.

Thus some of the characteristics of diploid and multiple honeybee spermatozoa may be found separately in other groups of insects.

Perhaps diploid honeybee drones may be considered more primitive than haploid ones.

Conclusions

The main difference between diploid and haploid spermatozoa is that the diploids, as well as most of their parts, are bigger. Internally, they are very similar except for the direction of the grooves on the mitochondrial derivatives.

Diploid drones normally produce multiple as well as single spermatozoa. These are larger than the single ones. The number of axonemes and mitochondrial derivatives is multiplied in multiple spermatozoa, but attachment of two derivatives to each axoneme does not exist in them. Multiple spermatozoa are not formed by fusion of separate sperms and probably develop from unpartitioned spermatocytes.

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