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Polymorphic Spermatozoa in the Hymenopterous Wasp Dahlbominus

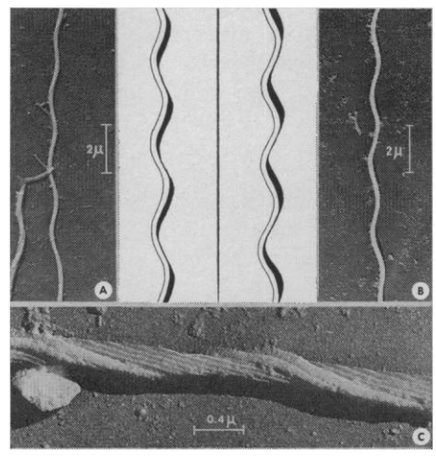
Abstract. *Studies with the light and electron microscope reveal that at least five different types of spermatozoa are produced during spermatogenesis in the arrhenotokous hymenopteran* Dahlbominus fusciupennis *(Zett. Eulophidae). Two of the types differ from the others in length, one type lacks a continuous spiral helix in the head piece, and two differ from the others in the direction of the helical coil. Few of the first three types reach the female sperm-storage organ. All the spermatozoa of the last two types that do reach the storage organ have heads with either dextral or sinistral helices extending from the apex of the head to the beginning of the tail piece. The mitochondrial filaments of the tail are also spirally coiled around the central axial filament, but only in a dextral direction. It is suggested that the coiling dimorphism may be related to fertilization of the egg and may thus affect the sex ratio.*

It is now generally recognized that not all products of spermatogenesis are alike or regularly functional. Among insects, aberrant spermatozoa are produced regularly in a number of families, classical examples being those reported by Schrader and others in the Pentatomidae (*1*). In species of this family, spermatozoa larger and smaller than normal, and with variable numbers of chromosomes, are produced by the "harlequin" lobe of the testis. Giant spermatozoa have also been found regularly among normal-sized spermatozoa in certain stick insects (*2*) and the American cockroach (*3*). Most of the polymorphism reported so far has been related to size, and it is doubtful whether the aberrant spermatozoa produce viable offspring. Thus they would appear to have little direct influence on the heredity of the species. Aberrant spermatozoa have not been reported in the Hymenoptera where, in most species, femaleness is dependent on fertilization of the egg. In these species, nonfunctional spermatozoa could have direct effect on the sex ratio and on subsequent inheritance, provided that polyspermy was infrequent.

Recent studies (*4*) of the chalcid wasp *Dahlbominus fuscipennis* (Zett.), in which dispermy of the egg is rarely greater than 2 percent, revealed that males of this arrhenotokous species regularly produce spermatozoa of several aberrant types, at least two of which do not relate to size. Under the light microscope, the normal spermatozoa appear thread-like, with distinctive corkscrew-like heads and long, attenuated tail-pieces. The mean overall length of 194 spermatozoa removed from the spermathecae of several females was 189.05 ± 1.98 μ; of the spiral head piece, 30.82 ± 0.23 μ, the sharply pointed, spirally convoluted head being consistently about one-sixth of the total length. In the seminal vesicle of the male, however, variability in total length, and coiling of the head of the spermatozoa, were very pronounced. Some were much shorter and others longer than normal; some lacked the usual spiral coiling of the head (the heads gave a positive reaction with Feulgen's stain); such spermatozoa constituted about 7, 8, and 26 percent, respectively, of the spermatozoa in the seminal vesicle. However, none of the long or short spermatozoa and very few of the noncoiled spermatozoa (20 of 2754 examined) were found in the female storage organ or spermathecal capsule. Inseminated females invariably contain in their spermathecae spermatozoa uniform in size and degree of coiling.

Studies with the phase-contrast or light microscope revealed that the helical coils of the head pieces of spermatozoa from the seminal vesicle of the male and the spermatheca of the female are of two types. In some, the coil is directed clockwise; in others, counterclockwise. When carefully focused under a light microscope at × 1600, on either the upper or lower surface of the spermatozoa, the lines of the helices in focus can be seen to run in opposite directions. The proportion of spermatozoa with dextral and sinistral coils has thus been determined and the findings will be reported elsewhere (*5*).

To confirm the presence and precise nature of the dimorphic coiling, we have made electron micrographs of spermatozoa from the seminal vesicle of mature adult males; the vesicles were dissected from a number of freshly killed insects and the sperma-



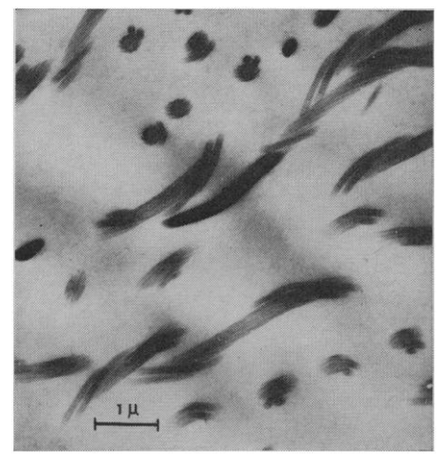


Fig. 1 (left). Electron micrographs of spermatozoa shadow-cast with palladium. (*A*) Head dextrally coiled, as indicated by the prominent diagonal shadows from left to right and as emphasized in the accompanying diagram. (*B*) Head sinistrally coiled, with the diagonal shadows from right to left. Sinistral coil illustrated in diagram at left of micrograph. (*C*) Part of a tail piece showing the dextral direction of the helix formed by the coiling of the double mitochondrial filaments around the central axial filament complex. Fig. 2 (right). Electron micrographs of sections of sperm in the male seminal vesicle. Sections of the head are electron-opaque, and ridged double strands of the mitochondrial filaments surround the axial filament complex.

tozoa were dispersed by ultrasonic treatment in water distilled in glass. Minute drops of the suspension were placed on Formvar-coated grids and allowed to dry in air. The grids were shadow-cast with palladium. The vesicles of the other males were fixed in 1 percent osmic acid buffered with Palade's solution (*6*), dehydrated in ethyl alcohol, and embedded in Epon according to Luft (*7*). Sections were stained with an aqueous solution of uranyl acetate to intensify contrast. Micrographs were obtained by means of a Siemens Elmiskop I (*8*).

Two basic types of spermatozoa are shown in Fig. 1. The gyres in some are directed dextrally (Fig. 1*A*); in others they are sinistral (Fig. 1*B*). Differences in the directions of the gyres occur only in the head, however; the gyre of the tail piece is always dextral (Fig. 1*C*). The central axial filament complex of the tail piece (see Fig. 2), which is of the usual type, is crossed at intervals of approximately 8.9 (one interval being the ratio of the diameter of the axial filament to the distance between cross-over points) by the two mitochondrial bands coiled dextrally around it. In spermatozoa with dextrally coiled heads the gyre continues uninterrupted from the tip of the head to the end of the tail piece, whereas in spermatozoa with sinistrally coiled heads the gyre is reversed in the tail piece.

There is some evidence that production of the two types of spermatozoa is related to the sex ratio of the offspring. When the proportion of the two types of spermatozoa in the female spermatheca is compared with the sex ratio of the progeny produced by the females, a much lower proportion of spermatozoa with dextrally coiled heads always occurs in the sex-ratio strain which has few females than in wild stock which has many females. In a series of tests with females of strains producing low proportions of females, 5 percent of the offspring being female, the proportion of dextral to sinistral spermatozoa in their spermathecae was 387 to 627, or 38 percent dextral; with females from wild stock, where 90 percent of the offspring were female, the proportion was 998 to 421, or 70 percent dextral.

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Electrical Connections between Visual Cells in the Ommatidium of Limulus

Abstract. *When microelectrodes are inserted in two cells of the same ommatidium in* Limulus, *current applied through the membrane of one cell produces a change in potential across the membrane of both cells. The large spikes recorded from one cell and the small spikes recorded from the other always appear synchronously. However, the small spikes are abolished selectively during the hyperpolarizing response. The discrete waves recorded from eyes adapted to darkness occur simultaneously in cells of the same ommatidium.*

The lateral eye of *Limulus* consists of several hundred ommatidia, each of which contains about 12 retinular cells and one or occasionally two eccentric cells. An elaborate structure of microvilli formed by the membrane of retinular cells, the rhabdom, is thought to contain a photosensitive substance and to initiate the process leading to vision. Eccentric cells possess a fairly large axon, from which impulses can be recorded following illumination (*1*). Smaller axons in the optic nerve are supposed to originate from retinular cells, but no one has succeeded in recording activity from them (*2*). When a microelectrode is inserted in an ommatidium, a negative potential is frequently recorded, suggesting penetration of an ommatidial cell. In darkness, the potential difference between the intracellular electrode and the outside is around −50 mv. This potential is decreased (and in some cells it can even be transiently reversed) during illumination.

In the majority of penetrated cells, small spikes can be recorded superimposed on the slow potential. In a smaller number of cells, the spikes are considerably larger. MacNichol (*3*) and Tomita *et al.* (*4*) have suggested that units of the first type are retinular cells and the others are eccentric cells (*3*, *4*).

In agreement with the observation that no activity can be recorded from the small optic nerve fibers, it has been suggested that the small spikes recorded with microelectrodes are not due to impulses originating in the retinular cells themselves but are reflections of the impulses of the adjacent eccentric cell. This interpretation implies that the two types of cells are electrically connected, as suggested by Tomita *et al.* (*4*).

In our work, two electrodes were inserted in the same ommatidium as done previously by Tomita and Miller (*5*) and by Behrens and Wulff (*6*). By means of a carefully balanced Wheatstone bridge similar to that described by Frank and Fuortes (*7*), it was possible to pass currents through either electrode while recording potentials simultaneously from both.

In most experiments, one electrode was placed in a cell producing large spikes (eccentric) and the other in a cell giving only small spikes (retinular), as shown in Fig. 1*d*. The currents applied through one electrode produced a change in potential in both cells. Similar results were obtained when the two microelectrodes were placed in two retinular cells in the same ommatidium. However, no electrical interaction could be detected when the two electrodes were placed in different ommatidia.

Figure 1*a* shows the result obtained when a bright flash of light was applied to the eye. Electrode 1 recorded a small slow potential and large spikes while electrode 2 recorded a larger slow potential but only barely detectable spikes. Large and small spikes were always synchronous. Similar results were obtained in all other pairs of cells studied. In the experiment illustrated in Fig. 1*b*, a current applied through electrode 2 evoked hyperpolarization of both cells. Reversal of the current depolarized both cells and evoked firing as illustrated in Fig. 1*c*; large spikes were recorded by electrode 1 and synchronous small spikes by electrode 2.

In many instances, the hyperpolarization evoked by applied currents developed in two steps, the first at the onset of the current and the second after some delay. The delayed change in potential is similar to that observed in other cells and is referred to as the hyperpolarizing response. It is due