

## A Comparative Study of Spermatozoa in Relation to the Classification of Mealybugs

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To offer proof of its validity, a system of insect classification must remain in agreement with the majority of facts uncovered during the continuous process of entomological research. Unless such a system is founded on a large area of empirical truth it will soon yield to a new and better concept. As a result of being subjected to a constant critical analysis there seems to be evolving slowly a genuine system of insect classification which should not prove as transitory as its predecessors. Such relative stability is being achieved only by using as the basis of classification a correlation of the best information available from all sources, morphological, physiological and ecological.

One purpose of this paper is to cite an example of how perhaps seemingly irrelevant material may at times contribute to the science of systematics. For although this paper had its origin in an anatomical study of insect spermatozoa, its findings are now employed to add supporting evidence to a recent revision of the genus *Pseudococcus* based on entirely different characters.

The technique for examining spermatozoa of mealybugs is remarkably rapid and relatively simple. The testes are dissected from a male mealybug and placed in a small droplet of distilled water on a clean glass slide. The testes are then ruptured with a needle to free the spermatozoa into the water droplet. In order to keep the slides as free of extraneous material as possible, it has been found advisable to make a transfer of about half the spermatozoa to another water droplet by means of a micropipette improvised from a finely drawn-out piece of glass tubing. This method of transferring mealybug spermatozoa has also been successfully employed in the preparation of grid mounts for electron microscopy. The water droplet containing the spermatozoa is allowed to dry, and it is a fortunate and remarkable fact that the spermatozoa will withstand not only dessication but also the vacuum of an electron microscope without any apparent alteration of form. If the spermatozoa are to be examined with a conventional compound light microscope they are stained, after drying, with giemsa blood stain and then rinsed in distilled water. Other stains are satisfactory, but the rapidity of the giemsa blood stain method (2 minutes) gives it a decided preference. This entire technique, including the dissection of the male and the preparation and staining of the spermatozoa, can be accomplished easily within 10 minutes time if the drying process is hastened by warming the slides over a spirit lamp. The spermatozoa adhere tightly to the glass slides and do not require a mounting medium nor a cover slip. Prepara-

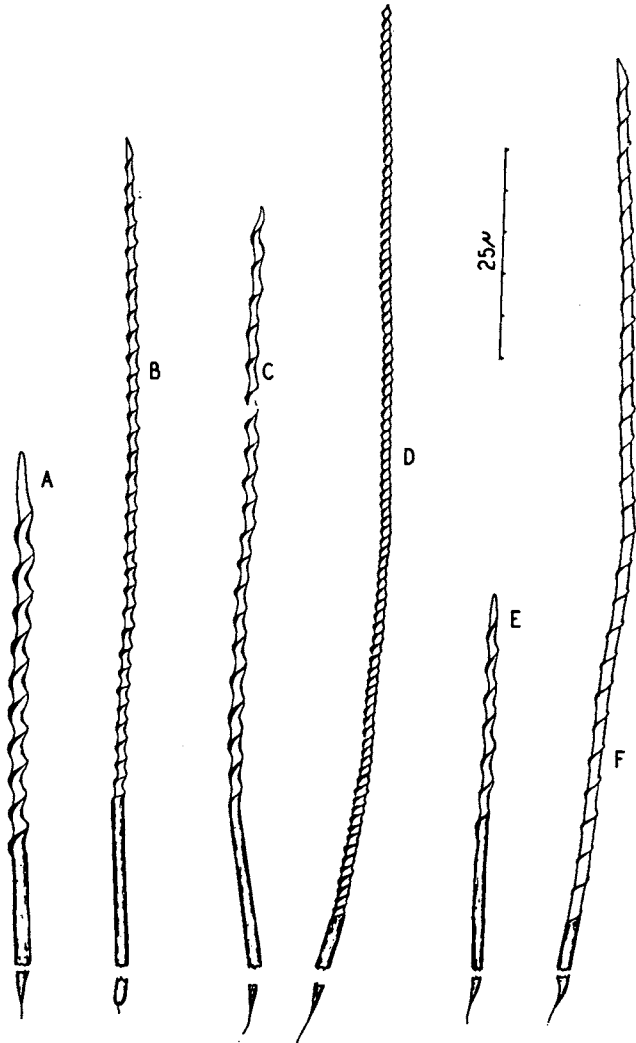


Figure 1. Diagram of mealybug spermatozoan heads and endpieces. A. *Spilococcus implicatus*; B. *Planococcus citri*; C. *Nipaecoccus aurilanatus*; D. *Pseudococcus maritimus*; E. *Dysmicoccus timberlakei*; F. *Phenacoccus gossypii*.

tions have remained without apparent change for over two years and may last indefinitely.

Photomicrography has been used extensively in the study of these spermatozoa. The line drawings of sperm heads in Figure 1 were made directly on matte-surfaced prints of photomicrographs. (The end pieces shown in the same figure are entirely diagrammatic, however.) In order to maintain true proportions and size relationships between species, the photomicrography in this investigation was standardized by using exactly the same magnification on the film and identical enlargements of negatives each time a spermatozoan type was studied. Normal illumination with a ribbon filament was used for the most part, although dark field illumination was helpful at times in demonstrating certain structures. Although electron microscopy also was used to advantage in disclosing the true nature of some of the smaller features, the mealybug spermatozoa are relatively opaque to the electron beam, and internal fibrillated structure is more readily studied with a conventional light microscope.

The spermatozoa of mealybugs seem to possess in common a basic morphological formula. This consists of a spiral or auger-shaped head followed by a long cylindrical flagellate tail composed of a cytoplasmic envelope surrounding a group of intertwined fibrils. This tail may constitute as much as 90 per cent of the total length of the spermatozoon and normally terminates in a slender filament, the endpiece. All of the first three species of mealybugs studied showed strikingly different variations of this basic pattern of spermatozoan form. This induced the publication of a preliminary note in which these interesting differences were illustrated and in which the possibility of employing spermatozoa as diagnostic tools in mealybug taxonomy was suggested (Doutt, 1949).

Subsequently, as more species of mealybugs were obtained for study it became evident that the differences between spermatozoa did not consistently hold at the species level as was earlier supposed. Instead, it was possible to group species having spermatozoa of similar appearance, and it was obvious that if the comparison of spermatozoan types was to have any meaning it would be in relation to a higher category than the species.

In 1950 Ferris revised the North American members of the genus *Pseudococcus* largely on the basis of knowledge which had accumulated from years of study of female mealybug morphology. In doing so he wrote the following paragraphs:

"Out of the species that have been referred to this genus in North America a number of genera will be made. The genus *Pseudococcus* itself will be reduced to less than 10 species. Unfortunately, in this process a few of the common and economically important species which have long passed under this generic name will be transferred to other genera, the most unfortunate of these changes involving *citri* and *brevipes*.

"How large the genus *Pseudococcus* will be when it has been adequately studied for the entire world remains to be seen. It is probable that some modification of the definition as here presented will be forced by such a study, but the examination of a considerable amount of ma-

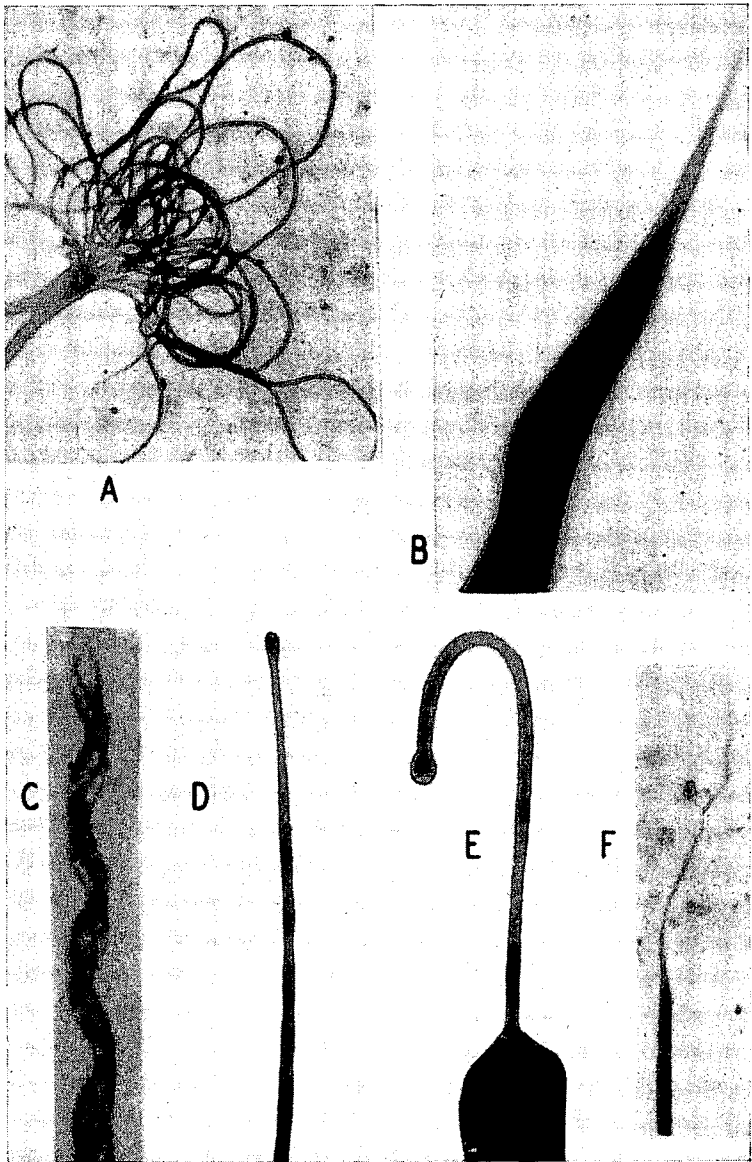


Figure 2. A. Fibrils fraying from broken spermatozoan tail of *Spilococcus implicatus*. B. Tip of spermatozoon of *Planococcus citri*. (Taken with electron microscope.) C. Intertwined fibrils of *Spilococcus implicatus*. D., E. Endpieces of *Planococcus citri* (Taken with electron microscope). F. Endpiece of *Spilococcus implicatus*.

terial from various parts of the world encourages the belief that the definition here presented will be basically sound."

A comparison of the spermatozoan types has been made with the new generic groupings as set forth by Ferris, and some interesting and probably significant parallelisms have been found to exist. With the exception of a single species (*brevipes*) among those examined, the categories of species based on spermatozoan structure fit exactly the generic scheme proposed by Ferris on the basis of female characters. The fact that the spermatozoa which have such a basic nature and function seem to fit Ferris' scheme of classification so closely adds considerable weight towards the proof of its validity.

On the basis of similarity of spermatozoa the following species group together: *adonidum*, *brevipes*,<sup>1</sup> *gahani*, what presumably is *maleacearum*, and all races of *maritimus*. With the notable exception of *brevipes* all of these species are retained by Ferris in the genus *Pseudococcus*.

Figure 1, D, illustrates the characteristic long and tightly spiralled head of the spermatozoon common to members of the genus *Pseudococcus*. This spermatozoon has a long tail which tapers to a filamentous endpiece which is illustrated somewhat diagrammatically in Fig. 1, D.

The spermatozoon of the common citrus mealybug exhibits features which immediately distinguish it from all other spermatozoa examined. It is interesting that Ferris recognizes this species, *citri*, as a member of a distinct new genus *Planococcus*. The head and the peculiar blunt termination of the tail are diagrammed in Fig. 1, B. The very fine, short endpiece is extremely difficult to photograph by means of a light microscope and this technique was successful only when dark field illumination was used. This endpiece is easily photographed by electron microscopy, Fig. 2, D, E. Examination by electron microscopy reveals a knob-like termination of the endpiece which is not seen with a light microscope, and it may be simply an artifact induced by the evacuated condition in the electron microscope. The tip end of the spiral head, which perhaps corresponds to the acrosome of mammalian sperm, is shown under electron microscopy, Fig. 2, B, to be far more elongate and pointed than is evident under ordinary microscopic examination.

The peculiar spermatozoon which was attributed to *Pseudococcus sequoiae* (Coleman) by Douthett (1949) has since been found to belong to a closely related but new species, described by Ferris as *Spilococcus implicatus*. The head and endpiece are shown diagrammatically in Fig. 1, A. This spermatozoon is somewhat more gross than spermatozoa of other species and is particularly suitable for demonstrating the internal fibrillated nature of the tail. When the cytoplasmic envelope of the tail is ruptured the enclosed fibrils fray out as is shown in the photomicrograph Fig. 2, A. The manner in which they are intertwined is shown in Fig. 2, C. The tapering tail and filamentous endpiece typical

<sup>1</sup> The author is grateful to K. S. Hagen who prepared mounts of spermatozoa from male *brevipes* supplied to him by the staff of the Pineapple Research Institute in Honolulu, Hawaii.

of most mealybug spermatozoa is pictured in Fig. 2, F. Quite often a vacuolated area appears in spermatozoa just anterior to the origin of the endpiece.

The golden mealybug, now considered to be *Nipaecoccus aurilanatus* (Maskell), possesses a spermatozoon different from other members of the old genus *Pseudococcus* with which it was formerly associated, Fig. 1, C.

An examination of spermatozoa from *Dysmicoccus timberlakei* (Cockerell) showed them to be quite distinct from any of the above genera, although the spermatozoan type is somewhat similar to *Spilococcus implicatus* Ferris. The spermatozoan type of *Dysmicoccus* has also been found in a number of unidentified mealybug males collected on filaree in the Coalinga foothill area of California. Ferris considers *brevipēs* to be a member of the genus *Dysmicoccus*, and as indicated above this is the only exception yet found to a perfect conformity between mealybug genera and spermatozoan types.

*Phenacoccus gossypii* Townsend and Cockerell has a spermatozoon of the type shown in Fig. 1, F. The head of this spermatozoon is quite long, being comparable to the length of the head possessed by spermatozoa of species in the present genus *Pseudococcus*. The core of the spiral head seems to be more definite in *Phenacoccus* than in the other genera studied and the spiral whorls seem to be somewhat superimposed on it. It is interesting to note that as great if not greater differences seem to exist between spermatozoan types within the members of the old concept of *Pseudococcus* than between some of these members and the long recognized genus *Phenacoccus*.

Thus on the basis of the specimens examined, with one noted exception, it would appear that distinct structural differences in spermatozoa serve to separate the mealybug genera *Pseudococcus*, *Planococcus*, *Spilococcus*, *Dysmicoccus*, *Nipaecoccus* and *Phenacoccus*.

It is of interest to note that such differences in spermatozoa do not exist with all insect groups. A great number of species of Coccinellidae have been examined, and in these beetles no difference in sperm structure is evident even between tribes. The coccinellid spermatozoon is similar to other coleopteran spermatozoa, but is extremely different from the spermatozoa of mealybugs. Coleopteran spermatozoa lack a spiral head and seem to be characterized by possessing a strong axial filament which supports an undulating membrane. In mealybugs the interwoven fibrils apparently contract in a time-sequence manner and thus impart a sinusoidal movement to the cylindrical flagellate tail.

**Summary.** A comparative study of mealybug spermatozoa has revealed that groups of species possess a common type of spermatozoon. With the exception of a single species among those examined, these categories of species based on spermatozoan similarities conform closely with the generic classification proposed by Ferris (1950). The fact that the structural types of such fundamental cells are so closely correlated with the grouping of species in the presently recognized genera adds

considerable evidence in support of Ferris' revision of the genus *Pseudococcus*.

LITERATURE CITED

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