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SIZE DIMORPHISM IN ADULT SPERMATOZOA OF ANASA TRISTIS.[1]

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I. Introduction.

The work on which this paper is based was undertaken with the object of determining the existence or non-existence of size dimorphism in the mature spermatozoa of *Anasa tristis*. Spermatogenesis studies of this form have shown that one half of the spermatozoa receive one more chromosome each than the other half. A corresponding difference in size of the mature spermatozoa may therefore be expected.

The study was carried on during the academic year 1912–1913, under the direction of Professor Charles Zeleny, to whom my thanks are due for his many valuable suggestions.

II. Materials and Methods.

1. *General Account.*—The material used in this study was collected from two localities, Urbana, Ill., and Bradford, N. H., during the month of October, 1912. The testes of these individuals were teased out in normal salt solution and made up into temporary and permanent smear preparations.

Smears of adult spermatozoa were prepared by the aceto-carmine method and by the osmic-hæmatoxylin method. In addition *intra vitam* staining was tried, but without success. The first mounts were made up as aceto-carmine preparations. Although this affords an excellent preparation for the study of spermatogenesis, it is not a good method to use in the study of adult spermatozoa. Curling is frequent. Distortion by swelling

[1] Contributions from the Zoölogical Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 23.

occurs more than is desirable. In the head of the spermatozoön no *inner chromatic rod* is differentiated from the cytoplasmic envelope. Moreover, it often takes several days to measure a considerable number of individuals, so that temporary preparations, such as those obtained with aceto-carmine, are objectionable. For this same reason *intra vitam* staining was abandoned.

For the most of the preparations Delafield's hæmatoxylin was used and proved much more successful than Schneider's aceto-carmine. The testis was taken out of the individual and placed in normal salt solution. It was then transferred to a slide that had been previously coated with a thin film of albumen fixative. The spermatozoa were teased out on the slide and examined in a drop of some fluid, such as Ringer's, to determine their motility or non-motility. After most of the solution had been allowed to evaporate so that only a bit of moisture was left around the spermatozoa, the preparation was fixed in osmic fumes for thirty seconds and then stained in Delafield's hæmatoxylin. This method was found to give a maximum number of straight spermatozoa with an *inner chromatic rod* well differentiated.

The microscope used in the measurements was equipped with a Leitz 2 mm. oil immersion objective and Zeiss compensating oculars. A large number of individuals was measured and a curve of variability plotted. In every individual it was the *length of the inner chromatic rod* which was determined. A description of this chromatic rod occurs later under the description of a mature spermatozoön.

2. *Sources of Error.*—The probable sources of error that need special consideration are (1) imperfect technique in preparations; (2) immaturity of the spermatozoa; or (3) incorrect measurement.

(1) *Error Due to Imperfect Technique in Preparations.*—Several smears of mature spermatozoa were prepared by the aceto-carmine method. Measurements made from them were not uniform, due to faulty technique. Many of the sperm heads had been curled or shrunken in fixation. Lack of an exact tip or base of the chromatic rod could have given rise to an error of from 1μ to 5μ in measurements of length. Distention and distortion were common. On account of the faulty prepara-

tions, the results obtained from them have not been included in the data.

In the aceto-carmine preparations it was found that drawing off the excess stain by a bit of filter paper as recommended, carried along with it many spermatozoa. In fact, preliminary measurements of such preparations showed a prevalence of larger spermatozoa on the side toward which the drawing was being made. Precautions were taken to eliminate this fault by fixing the spermatozoa to the slide with a thin film of albumen fixative. Also osmic fumes were used in preference to a liquid killing agent and Delafield's hæmatoxylin, instead of some more complicated stain, in order that all the spermatozoa might be preserved. This osmic-hæmatoxylin combination has been found particularly effective in producing straight spermatozoa with well-marked chromatic elements. In the preparations from which data were compiled from ninety-five to ninety-seven per cent. of the spermatozoa were sufficiently straight for measurement.

(2) *Error Due to Immaturity.*—When preliminary mounts were made about the first of October, 1912, the material was found to be too immature for the purpose of this work. All stages in spermatogenesis were present from the spermatogonium to the young spermatids, but no adult spermatozoa were found.

One of the problems confronting the writer in determining suitable preparations for study was the recognition of a truly mature spermatozoön. In general, the attenuated character of the head, with the chromatic rod (nuclear element) extending almost its entire length, is sufficient for the recognition of a mature spermatozoön. On that basis all preparations where more than five per cent. of the individuals were immature were discarded. In order to satisfy himself more fully of the maturity of the individuals measured, the author examined all of them in Ringer's solution, which demonstrated their motility. In this fluid the developing spermatids and immature spermatozoa were quiet, while the fully ripe spermatozoa were active for a considerable length of time. However, motility is not to be taken as a general criterion of maturity. In the house-fly, *Musca domestica*, for example, immature forms are motile. Again, these

spermatozoa were similar in all respects to those obtained at Woods Hole in August, 1913, from adult males of this species. Because of their structure and specific reaction the author is assured that all spermatozoa entering into consideration in the data are mature individuals.

The description of a mature spermatozoön becomes necessary at this point. As far as the writer has been able to discover no one has described a *chromatic rod* with cytoplasmic sheath for the mature spermatozoön of *Anasa tristis*. Paulmier's description and figures show no such structure. Meek's figure for the "penultimate stage" of maturing spermatozoa of *Stenobothrus viridulus* shows something of the nature of this chromatic rod (Plate III., Fig. 36).

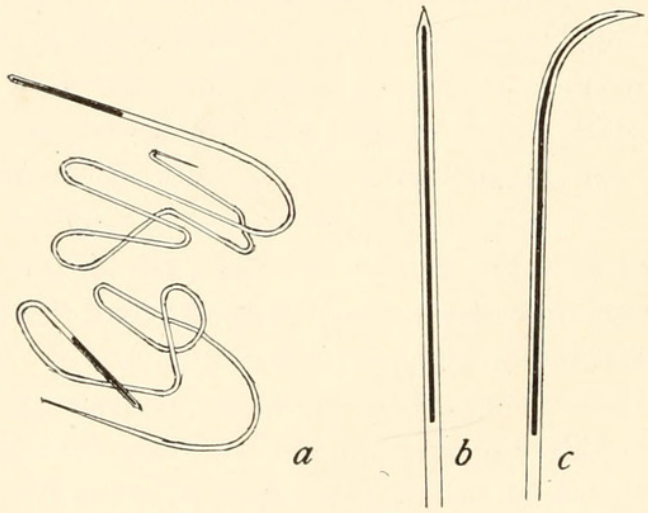

FIG. 1. Mature spermatozoa. (*a*) Entire spermatozoa; (*b*) straight sperm-head, the inner chromatic rod of this type was measured; (*c*) curved sperm-head, a characteristic type not measured.

The author's preparations agree with Paulmier's description of the mature spermatozoön of *Anasa tristis* in having no middle piece distinguishable, so that the tail connects directly with the posterior end of the head. The head has the chromatic elements compacted into a long attenuated rod of nearly constant diameter throughout its entire length. This *chromatic rod* is enveloped by a cytoplasmic capsule. The latter is also attenuated, and has a constant outer diameter. The length of the chromatic rod ranges from 24μ to 36μ and the width from $\frac{1}{3}$ μ to $\frac{2}{3}$ μ. Although the width is too minute for practical measurement, in general, it seems to be proportional to the length. The cytoplasmic

sheath is two to three times as thick as the chromatic rod. It extends 2μ to 3μ anterior to the anterior end of the chromatic rod, and is continuous posteriorly with the sheath of the axial filament. Considerable observation has shown that the frequency curve for chromatic rod length does not differ from the

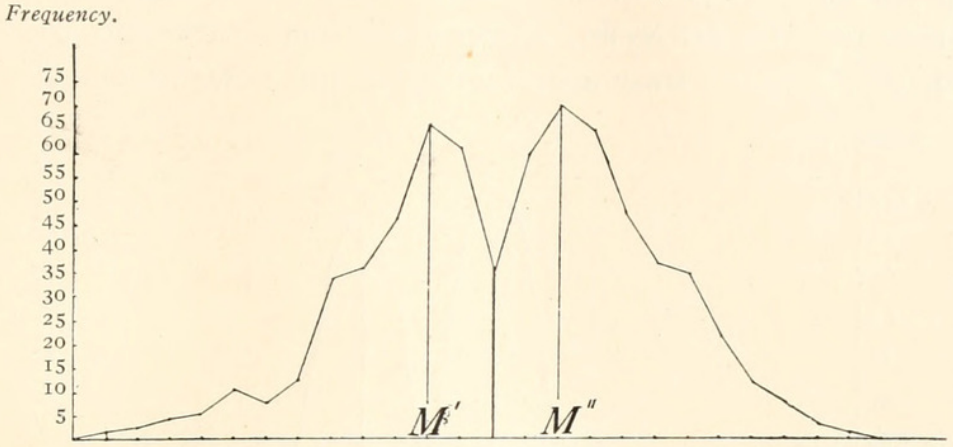

FIG. 2. Bimodal curve of variability of chromatic-rod length for 1r. Greater number of spermatozoa grouped around the upper mode, M''.

FIG. 3. Bimodal curve of variability of chromatic-rod length for 1l. Greater number of spermatozoa grouped around the lower mode, M'.

curve for the entire head. Unlike the adult spermatozoa described by Paulmier ('99) and Stevens ('05), the author's preparations show a *distinct region of cytoplasm around the chromatic rod*, and fail to demonstrate that the sheath is entirely, or even mostly "used as food material for the growth of the tail sheath," as Paulmier described it (p. 254; also Figs. 56 and 57). The author's hæmatoxylin preparations show no acrosome or

centrosome such as Paulmier described for this form. This difference might be claimed to exist because of the different staining capacities of Delafield's hæmatoxylin, used in the author's preparations, and iron-alum hæmatoxylin which Paulmier employed. However, Meek's preparations of the "antepenultimate stage" of *Stenobothrus*, which were stained with the iron-alum hæmatoxylin, show a distinct chromatic rod and sheath, but no acrosome and a diminishing centrosome.

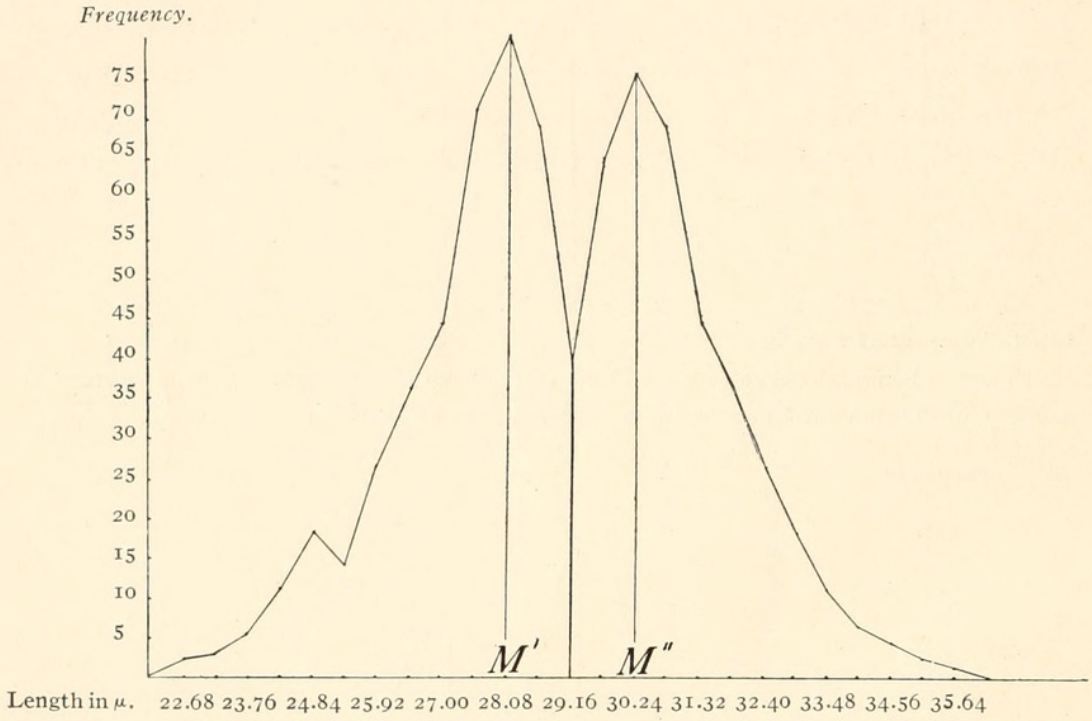

FIG. 4. Bimodal curve of variability of chromatic-rod length for 1r and 1l combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

Certain spermatozoa of unusually large size were observed. These are the so-called "giant spermatozoa." Such "giant spermatozoa" have been described by Henking ('91), and Wilcox ('95) for various species, and by Paulmier ('99) for *Anasa tristis*. Paulmier described those of "double and quadruple" the normal size, and attributed the phenomena "to the non-completion of one or both of the spermatocyte divisions." The author has observed only the double-sized forms. They are found in about one per cent. of the measurements. These spermatozoa are about one and one fourth times the length of the average normal spermatozoön, and contain about twice the amount of chro-

matic material of the normal forms. Smith's "giant spermatozoa" of hybrid pigeons, which are twice the normal length, do not appear to belong to this category. In a few cases the author has observed two mature chromatic rods of normal length within a single cytoplasmic membrane. Evidently these were cases where the chromatic elements had divided normally, but

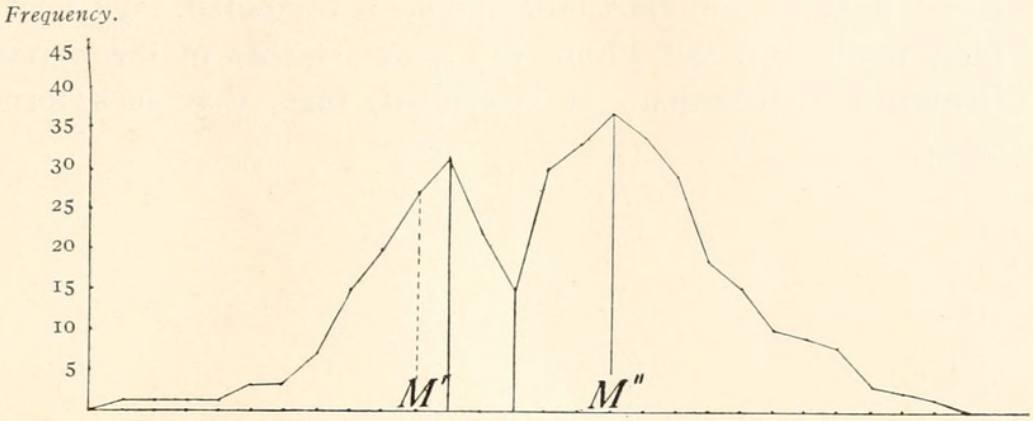

FIG. 5. Bimodal curve of variability of chromatic-rod length for 2r. Greater number of spermatozoa around the upper mode, M".

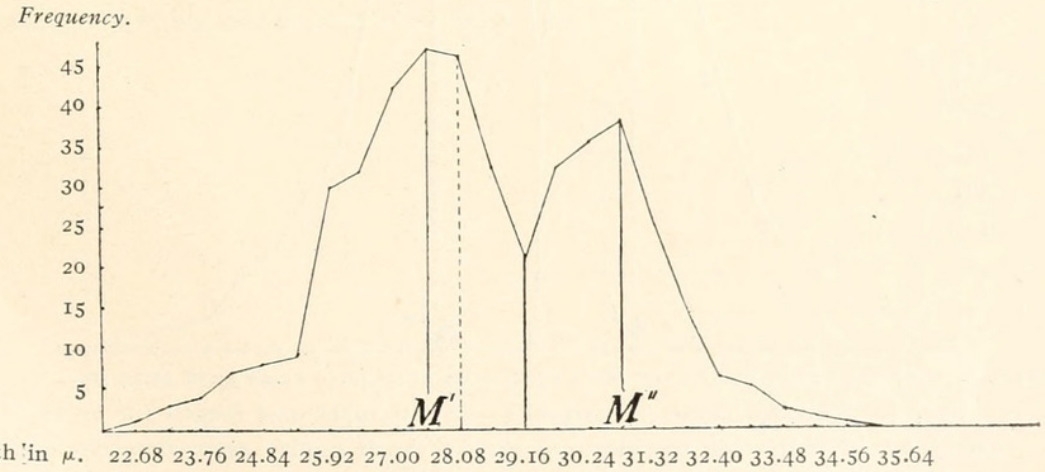

FIG. 6. Bimodal curve of variability of chromatic-rod length for 2l. Greater number of spermatozoa around the lower mode, M'.

where the cytoplasm had failed to divide, so that the two chromatic elements had developed together side by side within the same sheath. It is interesting to note that such "twin" spermatozoa are always of different lengths, varying from 1.5μ to 3μ from each other. "Giant spermatozoa" of this same type have been observed by the author in smear preparations of the housefly, *Musca domestica*.

The "giant spermatozoa" from the author's preparations had no definite range of variability but blended into the upper reaches of the curve for the normal spermatozoa. The most extreme "giant forms," as well as those that are less extreme, might be considered as wide variants in the frequency distribution of normal forms. If the average normal length is taken, and a "giant spermatozoön" of twice that chromatic volume is computed, then such a form would still fall within the upper reaches of the normal frequency distribution. It is evident, then, that such forms

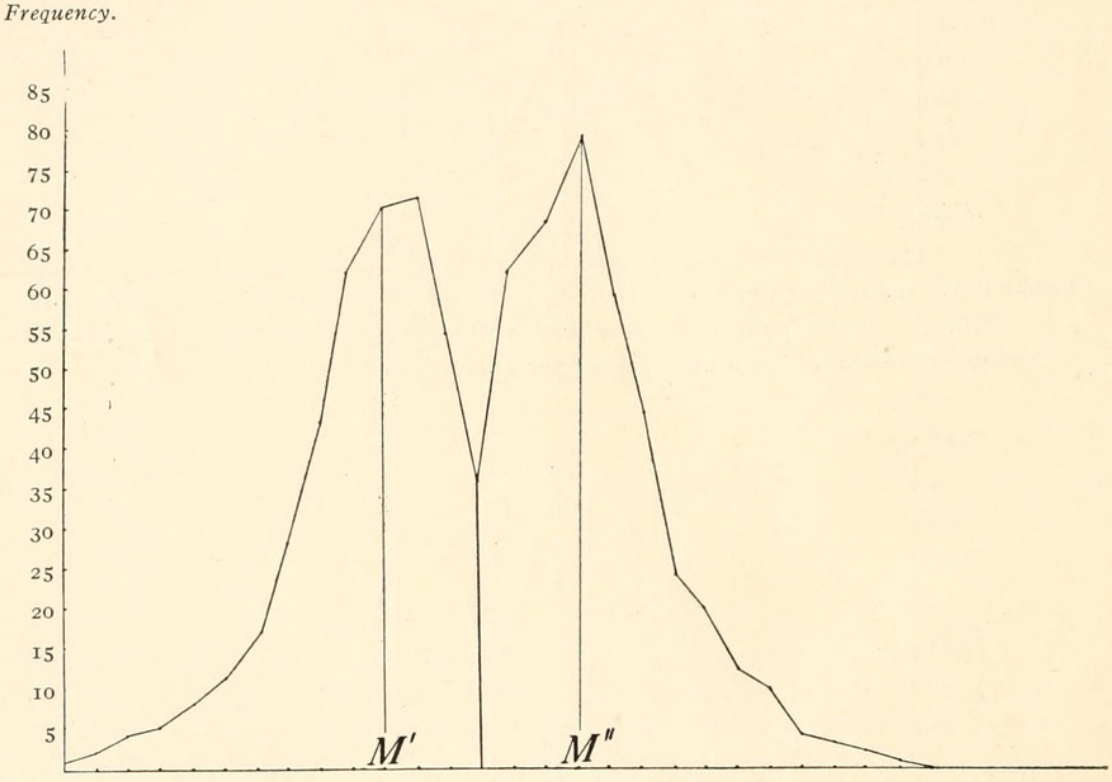

FIG. 7. Bimodal curve of variability of chromatic-rod length for 2r and 2l combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

can be considered as "giant spermatozoa" only when an arbitrary upper limit is placed to the normal frequency curve and these wide deviants considered as twice the volume of average spermatozoa.

(3) *Error Due to Incorrect Measurement.*—It is evident that, for the purpose of measurement, the inner chromatic rod must be well differentiated both anteriorly and posteriorly. Aceto-carmine preparation failed in this respect, while the Delafield's

brought it out clearly. Because the posterior limit of the chromatic rod is more easily located than the anterior limit, all measurements were made from the posterior end forward.

Another item to be taken into consideration is that the spermatozoön may not always be in a perfectly horizontal position on the slide, and hence all parts of it may not be in focus at once.

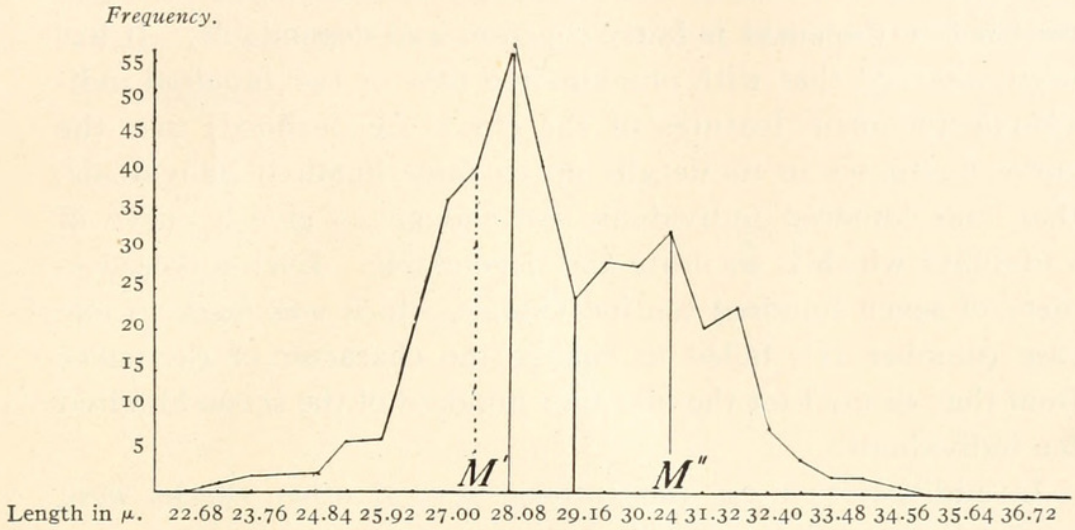

FIG. 8. Bimodal curve of variability of chromatic-rod length for 3r. Greater number of spermatozoa around lower mode, M′.

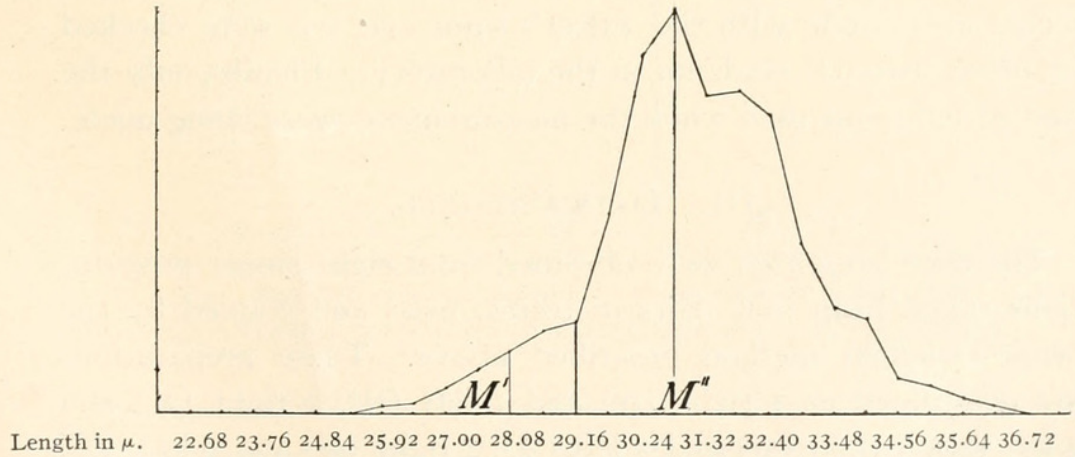

FIG. 9. Unimodal curve of variability of chromatic-rod length for 3l. M″ the only mode.

This may be due to a downward or upward bending or curving of the anterior end of the head, or to a tilting of the entire head, whether curved or straight. To preclude any danger of incorrect measurement all such spermatozoa were recorded separately and only entered in the data with those which were distinctly crooked.

The total of all spermatozoa, curved, crooked and tilted, which were not measured amounted to three to five per cent. of the numbers measured. Had this group been all of a critical length they would not have been sufficient in a single case to change the fundamental character of the curve of variability. An example of such a "curved" sperm-head is shown in Fig. 1, *c*.

A question arose as to how many spermatozoa must be measured before the curve is fairly constant and dependable. It has been observed that with one hundred fifty or two hundred individuals the main features of the curve are outlined; that the curve fluctuates in its details up to three hundred individuals; that four hundred individuals are enough to give a curve of variability which is accurate and dependable. Even a measurement of seven hundred ten individuals, which was made in one case (number 1r), failed to change the character of the curve from that secured for the first four hundred of the seven hundred ten individuals.

In addition to these precautions, several other checks were made in measurement. One preparation, number 1r, was measured three times with three different magnifications; the curves for all three measurements were similar. All measurements were made with the writer's same eye, but were checked by other research students in the laboratory. Finally, only the best of light was used while the measurements were being made.

III. Data Obtained.

The data presented were obtained from eight smear preparations taken from four pairs of testes, fixed and stained by the osmic-Delafield method described above. These preparations are designated as 1 right (1r) and 1 left (1l), 2 right (2r) and 2 left (2l), 3 right (3r) and 3 left (3l), 4 right (4r) and 4 left (4l). Taken as a whole the measurements show very clearly that there is a distinct size dimorphism in the spermatozoa. Individual testes, however, differ considerably from each other in details.

Preparations 1r and 1l.—These spermatozoa when examined in Ringer's solution were very active. From one thousand to two thousand individuals were present on each slide preparation, comprising the total number of spermatozoa from each testis.

With ocular no. 8, seven hundred ten individuals of 1r were measured. This number has been shown to be sufficiently large to plot a reliable frequency curve. Spermatozoa selected were taken as random samples from all parts of the slide. The variability curve is shown in Fig. 2. It shows two distinct modes at the lengths 28.08μ and 30.24μ, with a point of depression midway between at 29.16μ. Not only are the two modes equally distant from the intermediate low point, but the entire curve is

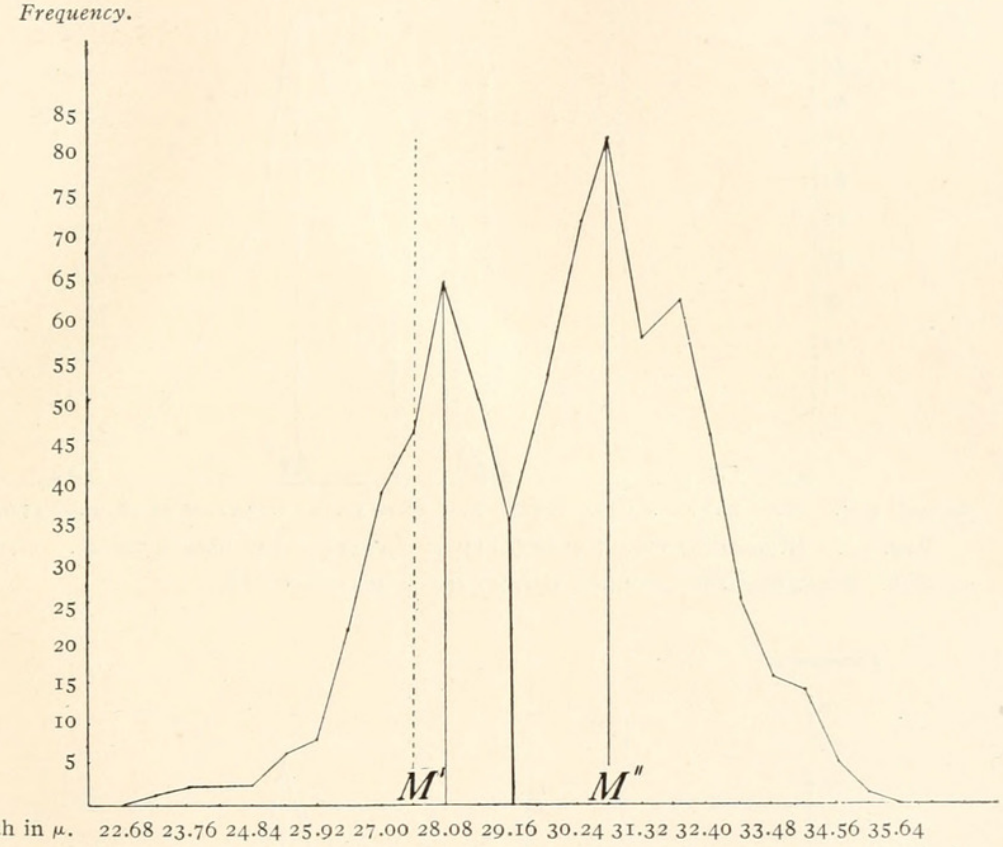

FIG. 10. Bimodal curve of variability of chromatic-rod length for 3r and 3l combined. Number of spermatozoa around upper mode, M″, is greater.

approximately bilaterally symmetrical. This same preparation was measured again with a no. 2 ocular and yet again with a no. 12 ocular. In the use of no. 2 ocular five hundred twenty-four individuals were measured; in the use of no. 12 ocular four hundred twenty-nine measurements were recorded. Both of these give curves agreeing closely to the bimodal curve of the first measurement.

With ocular no. 2, the magnification was hardly sufficient to correctly place the modes. Ocular no. 8 was found most satis-

factory and used in all the following measurements. For all these data each measurement was recorded in sequence and later incorporated into a polygon plot. All crooked or curved individuals were entered separately and not used except as a check.

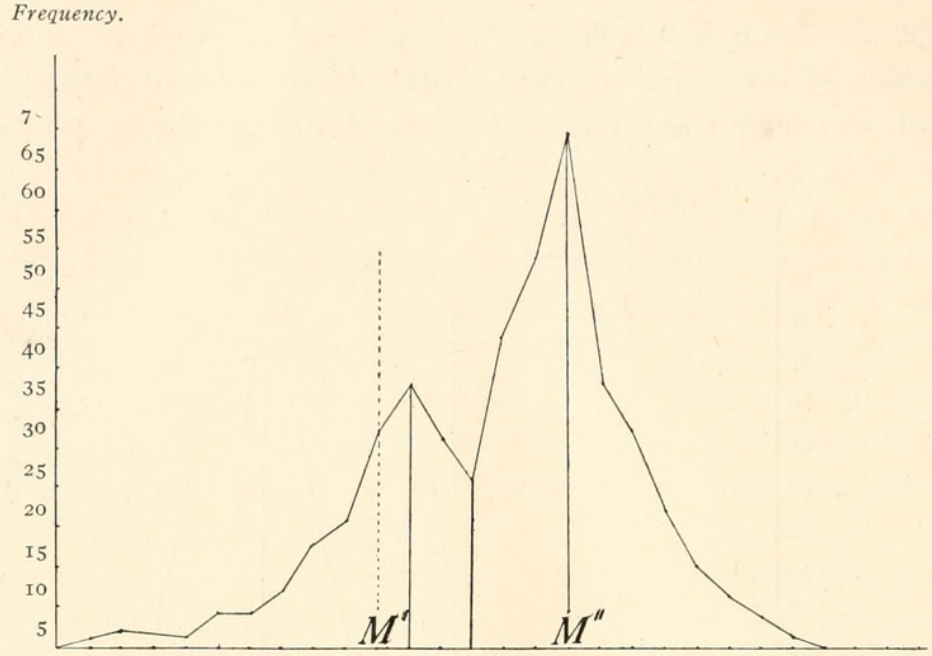

FIG. 11. Bimodal curve of variability of chromatic-rod length for 4r. Greater number of spermatozoa grouped around the upper mode, M''.

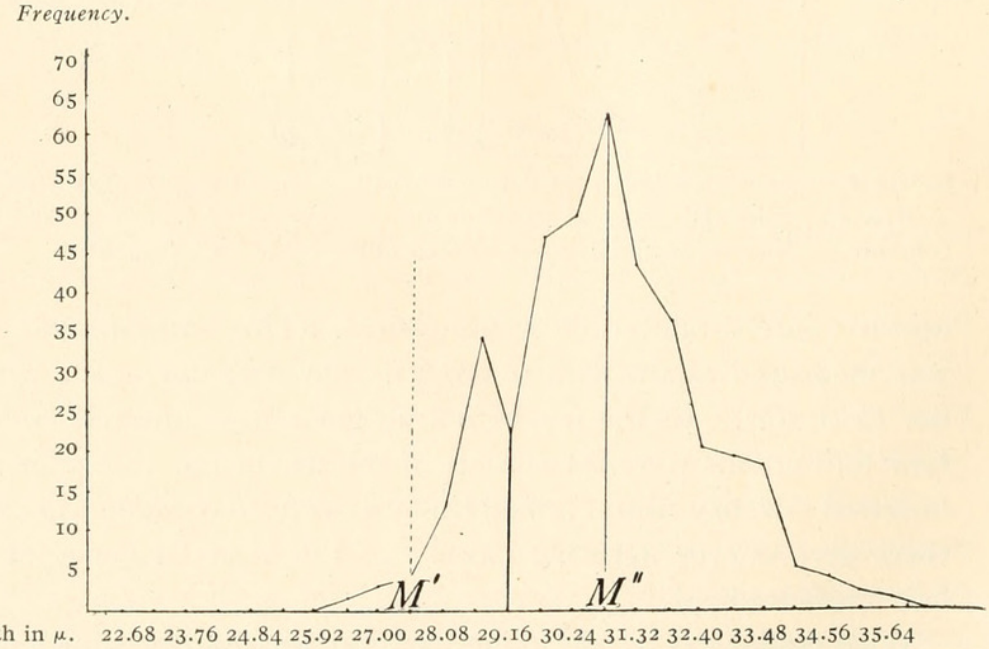

FIG. 12. Bimodal curve of variability of chromatic-rod length for 4l. Greater number of spermatozoa grouped around the upper mode, M''.

They amounted to 5.22 per cent. of the total number measured. Such a per cent. is not sufficient to affect the dimorphic character of the curve, even if it represented spermatozoa all of a critical length, *i. e.*, 29.16μ.

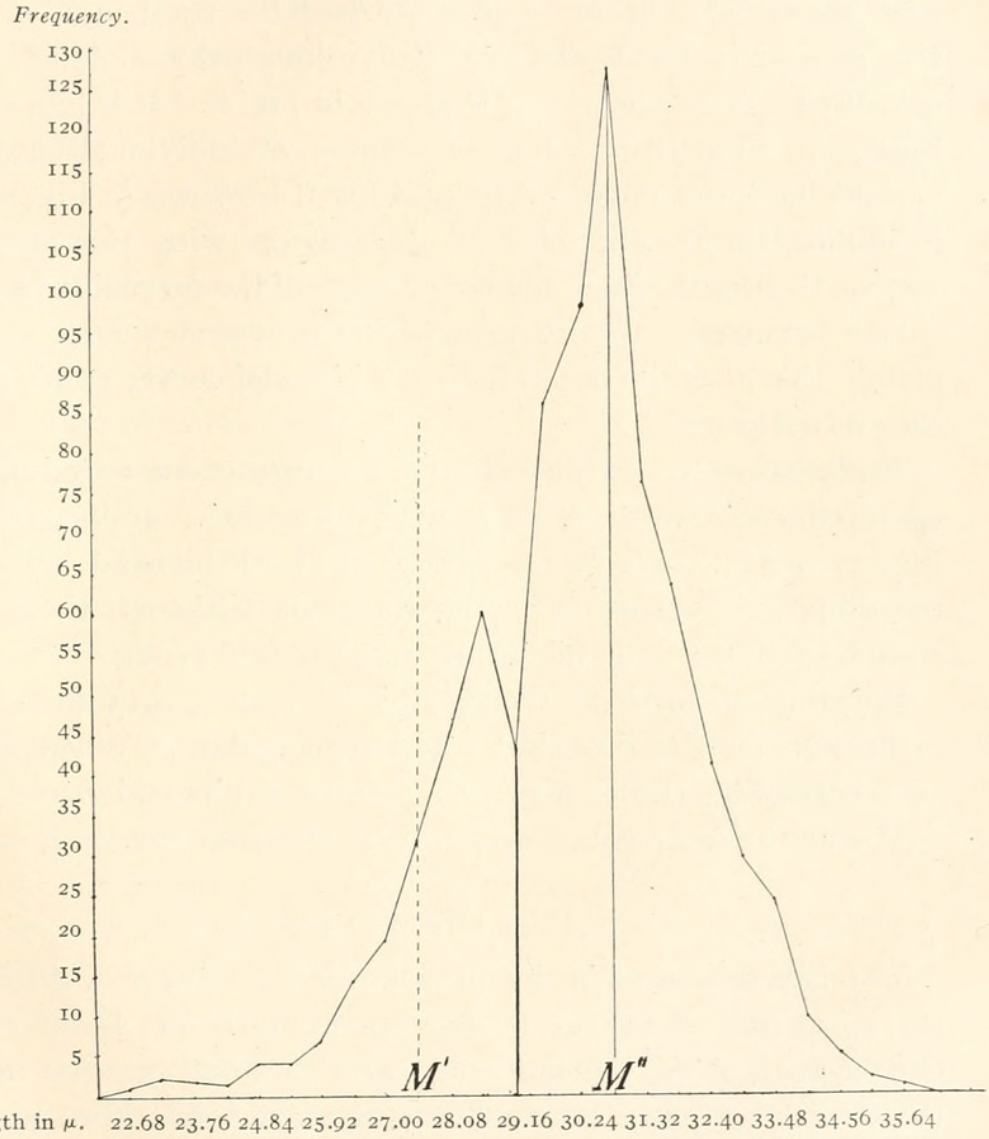

FIG. 13. Bimodal curve of variability of chromatic-rod length for 4r and 4l combined. Number of spermatozoa around upper mode, M'', is greater.

Four hundred individuals from 1l, chosen at random, were measured. This curve also shows a clear dimorphism. It is plotted in Fig. 3.

Preparations 2r and 2l.—Of the right testis 2r, four hundred eighty individuals were measured; of the left testis 2l, four hundred individuals were measured. The plots of these curves, Figs. 5 and 6, show that they are strikingly similar to those of

1r and 1l. It will be noticed, however, that 2r has 30.78μ as a mode of higher frequency, while 2l has 27.54μ as a mode of higher frequency.

Preparations 3r and 3l.—Of 3r only three hundred individuals were measured, because this comprised the total number that was present in the testis. Of 3l five hundred individuals were measured. The curve of 3r is shown in Fig. 8. It is bimodal in character, but with a greater number of individuals grouped around the lower mode. The plot for 3l is shown in Fig. 9. It is unmistakably unimodal in appearance, with the mode at 30.78μ, the length where the upper mode of the preceding bimodal curves occurred. Comparison with the bimodal curves shows plainly that it is the upper half of a bimodal curve, such as that shown in Fig. 2.

Preparations 4r and 4l.—In these preparations four hundred spermatozoa from each testis were measured. 4r is shown in Fig. 11, and 4l in Fig. 12. These are both bimodal and show the dimorphic nature of the spermatozoa, although they differ considerably from the bilaterally symmetrical type.

Preparations made at Woods Hole in August, 1913, from adult males were measured to check the foregoing data. When plotted as a variability curve, they, too, yielded a bimodal curve, with modes at the same lengths as those in the preparations 1r and 1l.

IV. Discussion.

The data presented in the previous section show very distinctly the dimorphic character of the spermatozoa of *Anasa tristis*. On the *basis of the chromatic-rod length* they fall into *two classes*, those with an extra amount of chromatin, and those without such an additional amount. Whatever differences and variations there are among the several curves, the differentiation into two groups remains proved. Such minor deviations as occur are probably due to secondary factors not yet discovered, and do not directly affect the bimodal grouping.

This pronounced dimorphic character of the spermatozoa of *Anasa tristis* must bear some fundamental relation to the dimorphism of chromosome number demonstrated by spermatogenesis studies. It is altogether probable that the mature

spermatozoa grouped around the higher mode of the curve of variability are those possessing the accessory chromosome, and that those grouped around the lower mode lack this accessory chromosome. The approximate equality of the two groups (see Fig. 2), moreover, strengthens the view that the two groups are produced as spermatids in equal numbers.

However, some of the curves do not show this approximate equality, but have marked digressions from a perfectly bilateral symmetry. They may have been produced as spermatids in equal numbers, and the departure from a bilateral grouping may be due to a difference in nourishment; or this deviation may be due to a slightly earlier ripening of the one group and their ejection from the testis.

Another interesting point brought out by the data is the relation between the right and left testis of the same individual. If the curves of 1r and 1l, 2r and 2l, are examined carefully, they are seen to approach bilateral symmetry even more closely when right and left testes of the same individual are plotted together than when each is plotted separately. Such combinations are produced in Figs. 4 and 7. The former is the combination curve for 1r and 1l; the latter is the combination curve for 2r and 2l. In the combination 1r and 1l, of eight hundred individuals plotted an actual difference of only eight is found between the two sides, or a difference of only one per cent. In the combination 2r and 2l, a difference of only five exists between the two sides of the curve, or a difference of only five eighths of one per cent. On the other hand combination curves for the testes of numbers 3 and 4 show no such close bilaterality. A probable explanation for this discrepancy is offered in the relation between the type of curve and abundance of spermatozoa. In the specimens numbers 1 and 2 there was a great abundance of spermatozoa in each testis. Because four to five hundred individuals were found to be reliable as a basis for a curve of variability, the entire number was not measured, but random samples were taken for measurement. In numbers 3 and 4, on the other hand, a different condition existed. For each of these there were not more than five hundred present in each testis. This abundance or scarcity of spermatozoa may have a very vital connection with the fundamental characters of the curve.

In the cases of most evident bilateral symmetry (in numbers 1r and 1l, 2r and 2l), the preparations were made up early in November, when the spermatozoa were still in abundance. In the cases where the curves do not show this bilateral symmetry (in numbers 3r and 3l, 4r and 4l), such a maximum number of mature individuals was not found. In one testis, at least, in each of these pairs (3r and 4r) the majority of the spermatozoa had been ejected from the testis. This fact was shown by the emptiness of the testis upon first examination. We may therefore provisionally consider the *normal condition* as one in which there is a *maximum number of mature spermatozoa*, and the *bilaterally symmetrical curve of variability* as the *best expression of such a norm*.

It might be assumed that this dimorphism in length is due not to any internal factor which marks out two groups of mature spermatozoa, but represents merely a sudden growth in length whereby the maturing spermatozoa pass over the low intermodal point very rapidly, so that few are "caught" in that place. There are definite reasons why such a theory cannot be accepted. The spermatozoa in the upper and lower reaches of the curve show by their structure that they are equally mature. This is shown not only by their general size and shape, but by their staining reaction which is very different from that of immature spermatozoa. Again, immature forms when they do occur are just as likely to occupy the low intermodal portion of the curve as the mature ones. In fact immature forms have been recorded for such a critical length. Such an objection must be put aside as unjustifiable.

V. Summary.

1. The work presented in this paper has proved the presence of size dimorphism in the adult spermatozoa of *Anasa tristis*. Measurements of the length of the chromatic rod of several hundred spermatozoa from each testis, plotted as a frequency curve, have demonstrated two modes, one between 27.54μ and 28.08μ, and the other between 30.24μ and 30.78μ, and the data offer adequate support for the conclusion that length dimorphism exists.

2. This dimorphism in length of adult spermatozoa probably bears a fundamental relation to the dimorphism in chromosome number revealed by spermatogenesis studies.

VI. BIBLIOGRAPHY.

Henking, H.

'91 Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insecten II. Zeit. f. wiss. Zool., Bd. LIV., 1892.

Meek, C. F. U.

'11 The Spermatogenesis of *Stenobothrus viridulatus*, with Special Reference to the Heterotropic Chromosome as a Sex Determinant in Grasshoppers. Jour. Linn. Soc., V., 32, pp. 1–22.

Paulmier, F. C.

'99 The Spermatogenesis of *Anasa tristis*. Jour. Morph., XV., Sup., pp. 223–272.

Smith, G.

'12 On Spermatogenesis and the Formation of Giant Spermatozoa in Hybrid Pigeons. Quar. Jour. Micr. Sc., V., 58, pt. 1, pp. 159–.

Stevens, N. M.

'05 Studies in Spermatogenesis. Pt. I. Carnegie Inst. Wash., Pub. No. 36.

Wilcox, E. V.

'95 Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. Bull. Mus. Comp. Zoöl., XXVII.