

CHROMOSOMES AND SEX DETERMINATION IN THE  
PARASITIC HYMENOPTERON *TELENOMUS FARIAI*  
(Lima)

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INTRODUCTION

THE problem of sex determination in Hymenoptera has attracted the attention of numerous investigators. Since in these insects, females are diploid and males are haploid, it has been thought for a long time that a single genome produces a male and two genomes a female. However, WHITING (1939, 1940a, 1943) and BOSTIAN (1939) have shown that in the parasitic wasp *Habrobracon juglandis* there exists a series of sex determining alleles xa, xb, xc, etc., any two of which produce a female (xaxb, xaxc, xbx, etc.), while a haploid organism carrying any one allele is a male. This mechanism of sex determination works best in a population having a variety of alleles, since in such a population diploid individuals homozygous for any one allele (xaxa, xbx, etc.) are seldom produced. Such homozygotes arise, however, in inbred strains and are less viable diploid males.

The present paper is concerned with sex determination in *Telenomus fariai*, a parasite of the eggs of the "barbeiro" bug, *Triatoma megista* (Burm). In contrast to *Habrobracon*, close inbreeding is the rule in *Telenomus fariai*. In *Telenomus*, daughters and sons of the same mother copulate before leaving the eggshell of the host. Despite the brother-sister mating, diploid males are unknown. This difference in the behavior of *Habrobracon* and *Telenomus* is in close relation with the remarkable cytological mechanism present in the latter form and described in the following pages.

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MATERIAL AND METHOD

*Telenomus fariai*, a representative of Celionidae, has been described by COSTA LIMA (1927). In another paper (1928), COSTA LIMA has pointed out the existence in this species of two classes of males, smaller ones which come from mothers which have been fertilized, and larger ones produced by virgin mothers. COSTA LIMA conjectured that the former are diploid and the latter haploid. TOLEDO PIZA (1929) has discussed the conjecture of COSTA LIMA, without, however, adducing any new facts. The present work was started originally with the intention of clarifying this problem of male dimorphism. Thanks to the kindness of DR. COSTA LIMA, we received two years ago some living material of *Telenomus*, which has been bred in our laboratory since then, and with which all our work has been done.

A detailed description of the biology of *Telenomus fariai* will be published elsewhere. The points essential for our present purposes are as follows. A fertilized female deposits a varying number of eggs (about seven or eight on the

average) into a single egg of *Triatoma*. The offspring of fertilized females consists of daughters and sons, always with a predominance of the former. Difficulties have been experienced in attempts to obtain virgin females; females which emerge from the eggs of the host proved to be nearly always impregnated, even if isolated immediately after their emergence. After much experience we have come to the conclusion that the only way to obtain virgins is to break the eggshell of the host and to isolate the females as soon as the latter hatch from the pupae. Indeed, wasps hatching from their pupae do not leave the eggshell of the host immediately, but remain inside for some time, move freely, and evidently copulate before emergence.

We can confirm the observation of COSTA LIMA (1928) that sons of virgin females are larger on the average than those of females which have been fertilized. The genetic constitution of both types of males, however, is the same; both always have ten chromosomes. The simple explanation of the dimorphism is as follows. Male larvae develop more slowly than the female ones; the latter consume more food and cause starvation of their more slowly growing masculine sibs. The offspring of virgin females consists of male larvae only; here the male larvae need not suffer from the competition of females and, though developing slowly, consume sufficient food to permit them to attain a greater average size than is attained by the sons of fertilized females. It must be noted, however, that while during the larval stage the growth of male larvae is slower than that of female larvae, during the pupal stage there occurs an inversion, and the males hatch from the pupae inside the egg of the host before their sisters. Although, as stated above, fertilized females produce fewer sons than daughters, a single male is sufficient to insure insemination of most or of all of his sisters inside the eggshell of the host. *Telenomus fariai* reproduces normally by brother-sister mating.

Acetic orcein, acetocarmine, and the liquids of Duboscq-Brasil and Gilson-Petrunkewitsch have been used as fixatives, acetocarmine, acetic orcein, iron haemotoxylin, and Feulgen as stains. Testes of adult males contain only spermatozoa; to study the early stages of the spermatogenesis, larvae and pupae have been used. The preparations were made by various methods: dissection of testes and ovaries, inclusion in paraffin, colorations in toto of the gonads, etc.

#### SOMATIC CHROMOSOMES

Figures 1 to 3 show the metaphase plates of somatic mitoses (chiefly of the nerve cells of larvae) in the females. The chromosome number is 20. A closer inspection discloses an important fact—namely, that the largest pair of V-shaped chromosomes consists of unequal partners. One of the V's is always equal-armed and the other is unequal-armed. The longer arm of the asymmetrical V is as long as either arm of the symmetrical V. This difference is always clear, although it is relatively more pronounced at the beginning of the metaphase when the chromosomes are less condensed (compare figures 1 and 2 with figure 3). There are two or three more pairs of V-shaped chromosomes and one pair of J-shaped ones, but no dimorphism is noticeable in these nor in the remaining rod-shaped ones.

Regardless of whether the offspring of fertilized or of virgin mothers is concerned, the somatic cells of the males have ten chromosomes (fig. 4-7). One of the ten is a large V-shaped chromosome, similar to the large equal-armed V in the females. In some more or less exceptional somatic cells chromosome numbers greater than ten are encountered; for example, figure 8 shows a cell with 18 chromosomes. This variation in chromosome number in somatic cells in Hymenoptera is well known and has been mistakenly interpreted as due to chromosome fragmentation. We know that not fragmentation but reduplication of some of the chromosomes owing to non-disjunction (polysomy) is here involved. Despite the variations, the chromosome number in the male is given as ten because (1) ten is the minimum and the most frequently observed number, and (2) the spermatogonia have always ten chromosomes. Some variations of the chromosome number have been observed also in the somatic cells of females.

#### SPERMATOGENESIS

The spermatogenesis in Hymenoptera has been studied by MEVES, DONCASTER, MARK and COPELAND, MEVES and DUESBERG, LAMS, GRANATA, ARMBRUSTER, NACHTSHEIM, WIEMAN, PATTERSON, TORVIK-GREB, and others (a review in SCHRADER and HUGHES-SCHRADER 1931). All these authors agree in general that one or both meiotic divisions are abortive. There are two variant types of spermatogenesis. The first, found in the honey bee, is characterized by the first division being abortive and resulting in the expulsion of a cytoplasmic bud without a nucleus, while a second division results in an equal division of the nucleus and a very unequal division of the cytoplasm, with the smaller of the two cells degenerating, and a single primary spermatocyte giving rise to a single spermatozoön. The second type, more common than the first and found for example in ants, entails also an abortive first division with an expulsion of a cytoplasmic bud without a nucleus, while the second division is equal, so that a single primary spermatocyte gives rise to two spermatozoa. *Telenomus fariai* represents a third variant type of spermatogenesis.

Figure 9 shows the chromosomes of an early spermatogonial mitosis; ten chromosomes may be counted, one of which is a large V with equal arms, similar to that found in male somatic cells. Figure 10 shows a mitosis of a very special kind observed solely in spermatogonia. The large equal-armed V-shaped chromosome seems to undergo an unequal division. This division results in formation of an equal-armed V which passes to one pole and an unequal-armed V which passes to the other pole. A notable fact is that one of the daughter cells derived from this division is much smaller than the other (fig. 11), does not divide further, and degenerates (fig. 12). Although it is not possible to verify by direct observation that the degenerating cell is the one which receives the large equal-armed V-shaped chromosome, this is made certain by the fact that in the spermatocytes the large V-shaped chromosome is always unequal-armed (fig. 13-15). The presence of small degenerating cells is always evident in young testes and may be observed also in older ones (fig. 16).

The unequal spermatogonial division is followed by a growth period (fig. 17) and the appearance of first spermatocytes, which may be distinguished

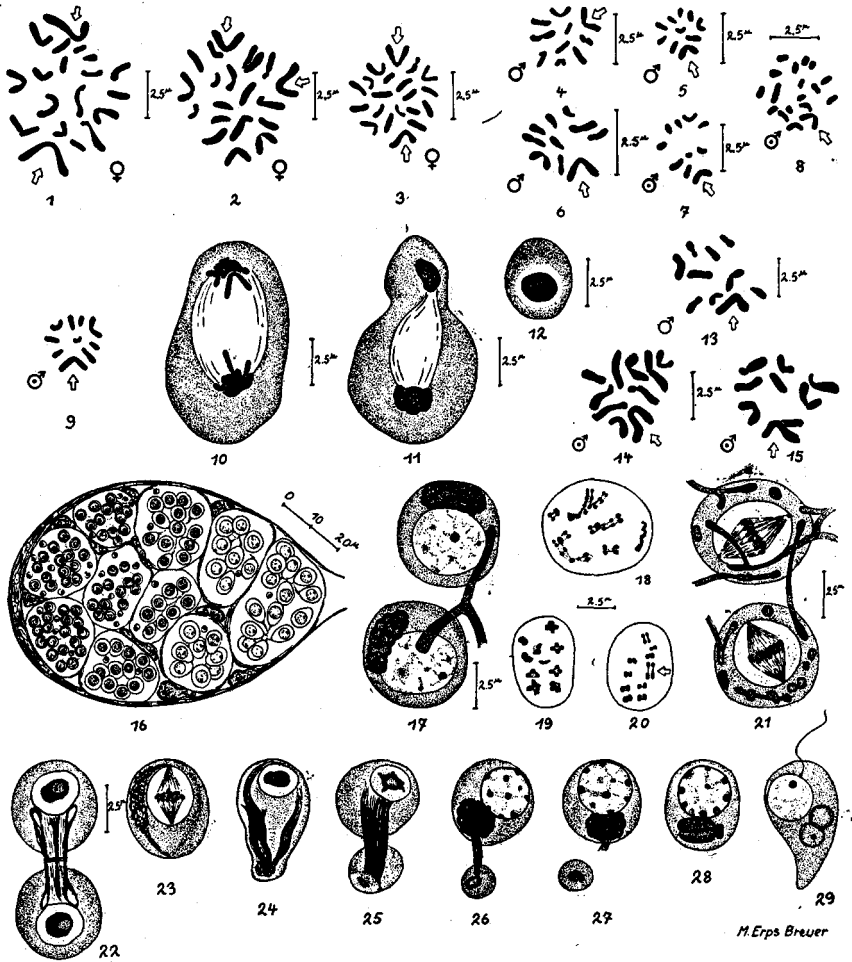


FIG. 1-3.—Nerve cells of ♀♀; arrows indicate the pair of V-shaped chromosomes consisting of unequal partners (X and Y). FIG. 4-6.—Nerve cells of ♂♂; arrows indicate the X chromosome. FIG. 7.—Somatic cell of a ♂. FIG. 8.—Somatic cell of a ♂ with 18 chromosomes. FIG. 9.—Spermatogonium of a ♂; the arrow indicates the X chromosome. FIG. 10-12.—Spermatogonia of ♂♂ undergoing the unequal mitosis. FIG. 13.—A spermatocyte I of a ♂; the arrow indicates a V-shaped chromosome with unequal arms (Y). FIG. 14, 15.—Spermatocytes I of ♂♂; arrows show the Y chromosomes. FIG. 16.—A section of a testis of a ♂, showing on the left spermatogonia and small degenerating cells and on the right spermatocytes and some degenerating cells. FIG. 17.—Growing spermatocytes I (♂). FIG. 18-20.—Prophase of the first division showing meiosis-like configurations of chromosomes. In Fig. 20 the arrow indicates the large V-shaped chromosome. FIG. 21.—Metaphase I. FIG. 22.—Telophase I (♂). FIG. 23.—Abortive division II in a ♂. FIG. 24.—Spermatocyte II of a ♂, beginning of the extrusion of cytoplasmic bud. FIG. 25-27.—Elimination of a cytoplasmic bud in a ♂. FIG. 28, 29.—First stages of spermiogenesis. ♂♂—sons of fertilized ♀♀; ♂♂—sons of virgin ♀♀. FIG. 1-4, 10-12.—Acetic orcein; 13-15.—Acetocarmine. FIG. 5-9.—Gilson-Petrunkewitsch, total preparations, Feulgen stain. FIG. 16-20.—Duboscq-Brasil, sections, iron haematoxylin.

from the spermatogonia by their large size. In contrast to other Hymenoptera so far studied, the first division of the spermatocytes in *Telenomus* does not result in expulsion of a cytoplasmic bud without a nucleus. Figures 18 to 20 show a prophase of this division in which the chromosomes assume shapes resembling meiotic bivalents. This, however, is an appearance only; the first metaphase shows ten chromosomes and is followed by a normal cell division and formation of two equal second spermatocytes (fig. 21, 22). The second division, on the contrary, is abortive and does not result in a nuclear division. A spindle is formed with a single centriole (fig. 23), an irregular metaphase plate appears in which one can see a large V-shaped unequal-armed chromosome, whereupon the nucleus reverts to the resting stage. A constriction appears in the cytoplasm on the side of the cell opposite to that containing the single nucleus, separating a globule which remains for some time attached to the cell from which it arose (fig. 24-27). Hence, in *Telenomus*, as in ants, two spermatozoa arise from each first spermatocyte, but the abortive division is the second and not the first. The process of spermiogenesis begins very soon after the expulsion of the cytoplasmic globule (fig. 28, 29). This is a further proof that the abortive division is the second and not the first. The behavior of the chondrioma ("Nebenkern") is easily observable and presents no peculiarities; here as in other Hymenoptera it resolves itself into elements which become arranged along the spindle (fig. 22) and which persist in the cytoplasmic bridges which join the cells together (fig. 17, 21).

#### OOGENESIS

The chromosome behavior in oogenesis is on the whole normal, except that the oocyte before the maturation divisions shows an intranuclear spindle which is sometimes perfectly regular (fig. 30, 30a), sometimes less regular, and sometimes even multipolar. This spindle regresses, and the nucleus reverts to its condensed state in which it persists for a long time while the oocyte grows (fig. 31). The meiotic divisions take place after the entrance of the spermatozoon into the egg (fig. 32-37). Four nuclei are formed (fig. 35), of which two fuse (fig. 36, 37) and eventually give rise to the so-called polar nuclei which become the trophamnion. Of the two remaining nuclei, one degenerates and the other (the female pronucleus) fuses with the nucleus of the spermatozoon (the male pronucleus). A study of the embryogenesis shows that in *Telenomus*, in contrast to what has been seen in related forms, there seems to be no visible differentiation of the cells of the germ track.

#### DISCUSSION

The classical work of BRIDGES has shown that in *Drosophila* the sex is determined by the relation between the number of X chromosomes and that of sets of autosomes. The situation in Hymenoptera does not fit this scheme, because here it seems as if the absolute number of chromosomes decides the sex of the offspring. The notable work of WHITING (1935a, 1935b) and of his students, especially of BOSTIAN (1934), however, has thrown a new light on this problem. In the parasitic wasp *Habrobracon juglandis* it has been shown that a female is always heterozygous (XY), while males are of two sorts (X and Y). Diploid homozygotes (XX and YY) are less viable diploid males. A similar scheme fits

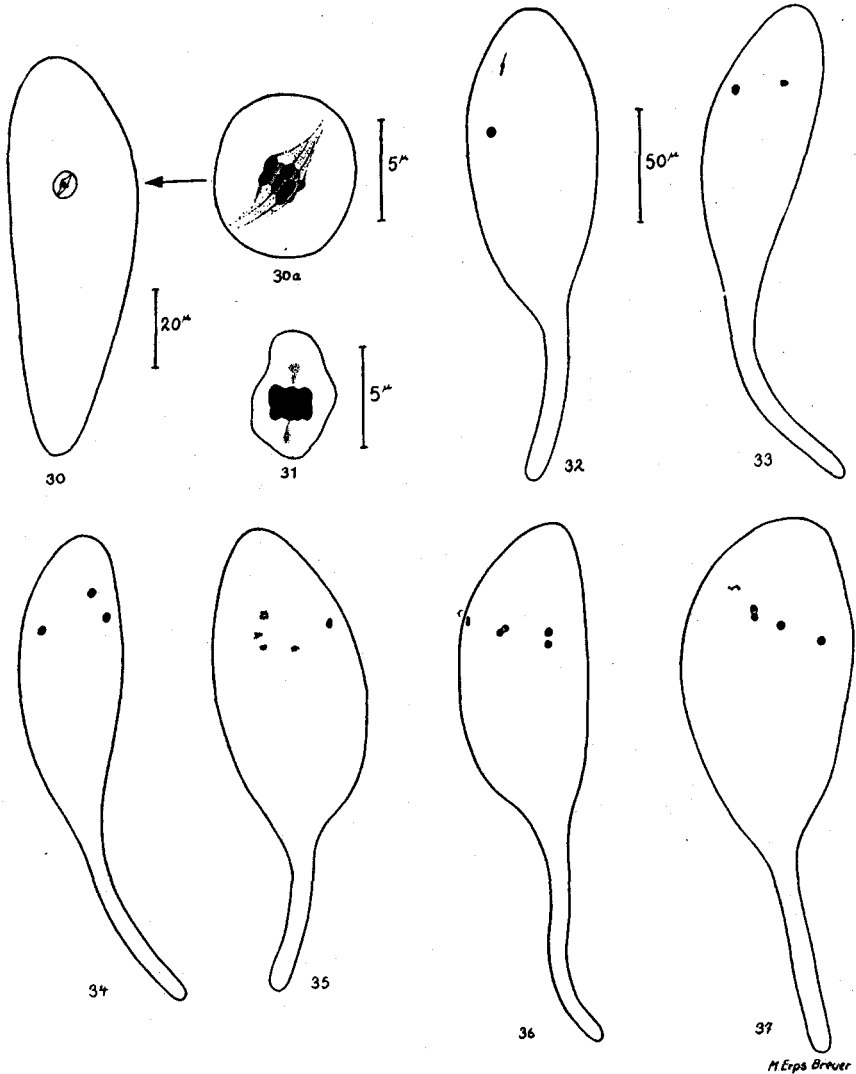


FIG. 30.—An abortive mitosis in a growing oocyte; a detail of the nucleus in Fig. 30a. FIG. 31.—A nucleus of an oocyte before fertilization. FIG. 32.—Fertilization. FIG. 33.—Fertilized oocytes; head of the spermatozoon transformed in ♂ pronucleus. FIG. 34, 35.—Meiotic divisions in oocytes. FIG. 36, 37.—Fusion of two polar nuclei. Gilson-Petrunkewitsch, total preparations, Feulgen.

also the case of another parasitic wasp, *Pteromalus puparum* studied by DOZORCEVA (1936a, 1936b). More recently, WHITING (1939, 1940a, 1943) and BOSTIAN (1939) came to the conclusion that in *Habrobracon* there is a multiplicity of alleles of the sex-determining locus in the X chromosome, and, therefore, many kinds of X chromosomes ( $X^1$ ,  $X^2$ ,  $X^3$ , etc.) occur in the species. Several kinds of diploid females and several kinds of haploid males are encountered. Indeed, with only six kinds of X chromosomes ( $X^1$ - $X^6$ ), 15 genetically distinct kinds of females may be produced. In a species in which outbreeding is the rule, there is a reasonable probability that a male which fer-

tilizes a female will have a different kind of X chromosome than the female has, and, therefore, no diploids having the same X chromosome twice will be produced. These diploids ( $X^1X^1$ ,  $X^2X^2$ , etc.) are known to be less viable diploid males. Evidently, the more sex-determining alleles a species has, the smaller the probability of the appearance of diploid males with outbreeding. Inbreeding, however, is followed by the production of diploid males. WHITING (1943) regards the different sex-determining chromosomes of *Habrobracon* as differing from each other in "differential chromosome segments" rather than in the allelic state of a single sex-determining locus, as thought earlier.

The situation known to exist in *Habrobracon* helps us to understand the biological meaning of some of the peculiarities of the chromosomal mechanism found in *Telenomus*. In contrast to *Habrobracon*, *Telenomus* reproduces mostly if not exclusively by brother-sister mating. According to the *Habrobracon* scheme, we might expect that 50 percent of diploid zygotes of *Telenomus* would develop into less viable diploid males, which are useless to the species. Natural selection could hardly permit a species so wasteful to survive. In reality, however, *Telenomus* seems to have developed the following mechanism which makes brother-sister mating compatible with non-production of diploid males. The female has a pair of large V-shaped chromosomes, one member of which is equal-armed and the other unequal-armed. We may call these chromosomes respectively X and Y. The somatic cells of the male and the earliest spermatogonia have always an equal-armed V-shaped chromosome (X), and this regardless of whether the male is a son of a fertilized or of an unfertilized female. We are forced to conclude that the process of maturation of the eggs is selective, in the sense that the functioning female pronucleus always carries the X chromosome. If so, the XY constitution of the female presupposes that the spermatozoa always carry a Y chromosome. How is it possible, however, for males which carry an X chromosome to produce spermatozoa with Y chromosome? The answer to this is apparently furnished by the unequal division of the early spermatogonia described above. These spermatogonia divide in two cells, one of which carries an equal-armed V (X) and the other an unequal-armed V (Y). It seems that during this process the X chromosome suffers an unequal division into two chromosomes, one of which is X and the other  $X-a=Y$ . The cells which receive the X chromosome degenerate and give the small spherules which eventually disappear. The cells which receive the Y are transformed into spermatocytes, undergo the further evolution described above, and give rise to functional spermatozoa, all of which carry a Y chromosome. Early spermatogonia show, therefore, an equal-armed V, and the spermatocytes an unequal-armed V. Owing to the technical difficulties, it has not been possible to demonstrate by direct observation that the female pronucleus always carries an X chromosome; this is inferred from the constitution of the somatic cells and of the early spermatogonia of the males.

#### SUMMARY

Fertilized females of the parasitic wasp *Telenomus fariai* produce offspring of both sexes, the males being small. Unfertilized females give rise to males only, but these males are large. The chromosome number in females is 20,

and in males, regardless of their origin, 10. The small size of sons of fertilized females is due to male larvae developing more slowly than their female sibs and consequently suffering from competition with the latter for food.

The female chromosome group contains a pair of large V-shaped chromosomes which consist of unequal partners: one chromosome is equal-armed and the other unequal-armed. The former is called the X and the latter the Y chromosome. Somatic cells and the early spermatogonia of the males have always the X chromosome. The spermatogonia suffer an unequal division, during which the X chromosome divides in such a way that an equal-armed V (X) passes to one and an unequal-armed V (Y) passes to the other pole. The cells which receive the X chromosome degenerate, and those receiving the Y become spermatocytes and are transformed eventually into Y-bearing spermatozoa. It is inferred that the Y chromosome differs from the X by the absence of a section of one of the two limbs ( $X - a = Y$ ). Oogenesis is normal; it is supposed that the female pronucleus has always an X chromosome. This remarkable chromosome behavior is presumably connected with the fact that *Telenomus fariai* reproduces normally by brother-sister mating. In other parasitic Hymenoptera, such as *Habrobracon*, inbreeding leads to production of some only slightly viable diploid males, which have never been observed in *Telenomus*.

The spermatogenesis in *Telenomus* differs from that in other Hymenoptera in that the first division of the spermatocyte is normal and gives rise to two second spermatocytes. The second division is abortive and leads to the expulsion of the enucleated bud of the cytoplasm.

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