OTHER METHODS OF SEX-DETERMINATION INVOLVING THE SEX-CHROMOSOMES

THE determination of sex through the redistribution of the sex-chromosomes in the germ-cells is regulated in some animals in other ways than those described in the preceding chapter.

FIG. 126.

Separation of the two small X-chromosomes from the autosomes in Ascaris eggs. (After Geinitz.)

The Attachment of the X-Chromosomes to Autosomes.

The attachment of the sex-chromosomes to other chromosomes, that is known to occur in a few forms, tends to conceal the differential character of X- and Y-chromo-

somes. Their presence has been detected, in such cases, by their occasional separation, as in Ascaris (Fig. 126), from their attachment, or by the differential staining properties of the X-chromosome in the male, or, as in certain moths studied by Seiler, by the regular separation of the compound chromosome into its components in the somatic cells of the embryo.
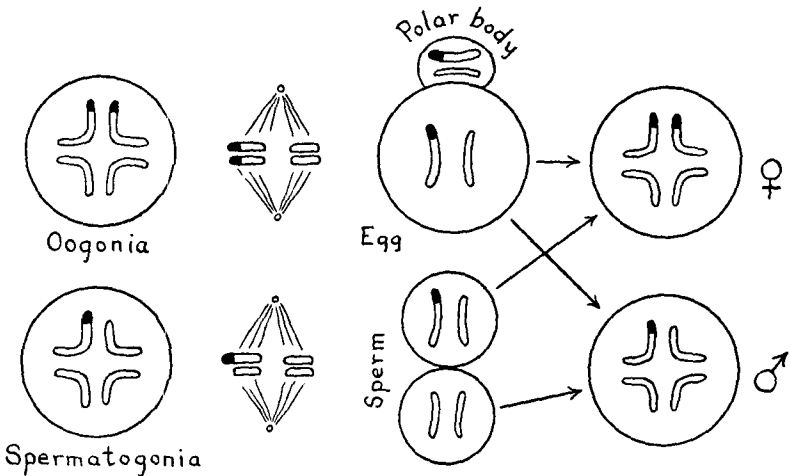

FIG. 127.

Diagram illustrating the distribution of the attached X-chromosomes in the male and female of Ascaris. (After Boveri.)

The attachment of the sex-chromosomes to ordinary chromosomes, or autosomes, as they are called, involves the mechanism of sex-linked inheritance, more particularly should crossing-over take place in the male between attached X. An example will illustrate the point at issue. the autosome attached to the X and its mate lacking the In Fig. 127 the X-chromosomes of Ascaris are indicated by the black ends of those chromosomes to which they are attached. In the female there are two X-chromosomes,

each attached to a member of the same pair of autosomes. In the mature egg one such compound chromosome (therefore one X) is left in each egg. In the male, one X is present, attached to the corresponding autosome, but the other autosome has no attached X. After maturation half of the sperm-cells will contain an X, half will be without an X. The mechanism for sex-determination is obviously here the same as in the XX-XO type.

In the female, crossing-over might take place both between the two X-chromosomes and between the two attached autosomes. But in the XO male the situation would be different; for in the male the X part of the compound chromosome has no opposite, hence no crossing-over is expected in that part. This would insure the coherence of the sex-differentiating genes and of the sex-mechanism; but between the autosomal parts of the compound chromosome an interchange might then take place without affecting the sex-mechanism. The characters whose genes lie in the X-component will show sex-linked inheritance, *i.e.*, the recessive character, will appear in the sons. The recessive characters whose genes are in the autosomal part will not appear in the sons. However, the character whose genes are in the autosomal part will show partial linkage to sex and to the characters whose genes lie in the X-component.[1]

In the imaginary example just given, the autosome without an attached X, that is, the mate of the compound chromosome with an X in the male, will appear to correspond to the Y-chromosome of the ordinary XX-XY type

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[1] According to McClung the X-chromosome in the male of Hesperotettix is not constantly attached to the same autosome, although in a given individual its attachment is constant. In other individuals it may be free. Were sex-linked characters known in such a type, their inheritance might be complicated by this inconstant relation of the X-chromosome to the autosomes.

(because it is confined to the male line), except, as just pointed out, that it carries genes that are like those in the corresponding part of the compound X-chromosomes. Cases of inheritance have, in fact, been recently recorded where certain genes appear to be carried by the Y-chromosome, and such cases have been interpreted to mean that the Y-chromosome itself may sometimes carry genes.

There is no objection to such a statement if interpreted as above, but there is an obvious objection to this statement if it is intended to mean more than this; for the chromosomal sex-mechanism would break down if the X and the Y of the male interchanged throughout. If this happened, the two chromosomes would after a time become identical, and the difference in balance that gives males and females would be lost.

The Y-Chromosome.

There are two groups in which the genetic evidence has been interpreted to mean that Mendelian factors may be carried in the Y-chromosome. In fish, belonging to two different families, it has been shown by Schmidt, Aida, and Winge that the Y carries genes. In the gypsy moth Goldschmidt has interpreted the result of species-crosses in the same way (here the W-chromosome). The latter results will be considered in the chapter on sex intergrades; the former may now be taken up.

In the small aquarium fish, Lebistes reticulatus, a native of the West Indies and northern South America, the males are highly colored and strikingly different from the females (Fig. 128). The females in different races are closely similar to each other, while the males show characteristic differences in color. Schmidt has found that when a male of one race is bred to a female of another race, the sons are like the father. If these hybrids (F_1) are inbred, their sons (F_2) are again all like the father,

and none of them show any characters of males of the maternal grandmother's race. The F_3 and F_4 males are again all like those of the paternal forefather. There seems to be here no Mendelian splitting for any characters that might have been expected to have come through the maternal grandparent.
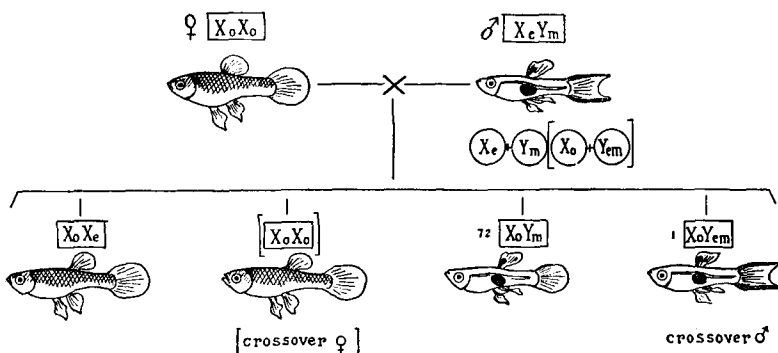

FIG. 128.

Diagram illustrating the inheritance of a sex-linked character in fish, carried both by the X- and the ''Y-chromosome.'' (After Winge.)

The same results are obtained when the reciprocal cross is made; the sons and grandsons are all like the paternal parent, etc.

In another fish, Aplocheilus latipes, inhabiting small streams and paddy fields of Japan, several types differing in color are found. Other types have also appeared in cultures. In these fish both males and females of each type occur. Aida has shown that several of these differences are transmitted through the sex-chromosomes (both X and Y). The genetic transmission of these characters can be explained on the hypothesis that the genes are carried sometimes in the Y- and sometimes in the X-

chromosomes, and that crossing-over between these chromosomes may take place.

For example, the character, white body color, is sex-linked in inheritance. Its allelomorph is red body color. When a pure white female was mated to a pure red male, the sons and daughters (F_1) were all red. When these were inbred they gave,

Red ♀	Red ♂	White ♀	White ♂
41	76	43	0

Assuming that the genes for white are carried in the two X-chromosomes of the female and designating such a chromosome by X^w (Fig. 129), and assuming that the

FIG. 129.

Diagram showing the inheritance of a character whose gene is carried by the "Y-chromosome," as well as by the X-chromosome.

gene for red is carried both by the X and the Y of the male, and designating these by X^r and Y^r, the cross given above works out on the XX-XY formula as shown in Fig. 129. If red (r) dominates white (w), both sons and daugh-

ters (F_1) will be red. If these are inbred the results are shown in the next diagram (Fig. 130). White and red daughters in equal numbers are expected and red sons only, that are equal in number to the sum of the two female classes.
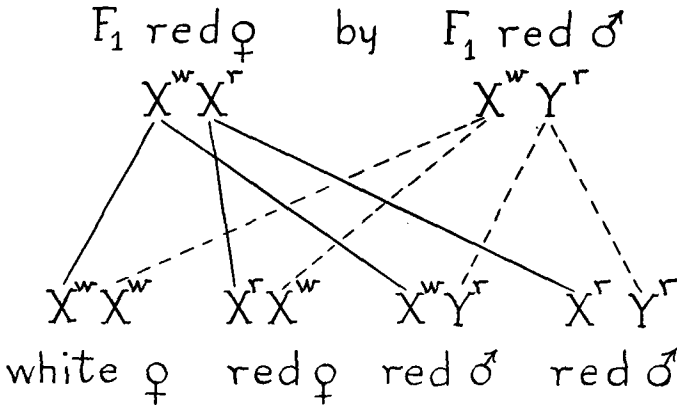
$$F_1 \text{ red } \female \quad \text{by} \quad F_1 \text{ red } \male$$
$$X^w X^r \qquad \qquad X^w Y^r$$

$$X^w X^w \quad X^r X^w \quad X^w Y^r \quad X^r Y^r$$
$$\text{white } \female \quad \text{red } \female \quad \text{red } \male \quad \text{red } \male$$

Fig. 130.

Diagram illustrating the inheritance of red and white color from two F_1 heterozygous male and female fish. The Y-chromosome as well as the X may carry the gene for red (r).

Thus from a red male and a white female no white grandsons are expected on this formulation unless in an $F_1 X^w Y^r$ red male, crossing-over between X and Y occurs to give a Y^w chromosome (Fig. 131). When such a chromosome meets an egg-carrying X^w, a white male, $X^w Y^w$, will be produced. A white male appeared, in fact, in one experiment in which an F_1 heterozygous red male, $X^w Y^r$ (obtained in the above experiment), was back-crossed to a pure white female. The results obtained were:

Red $\female$	White $\female$	Red $\male$	White $\male$
2	197	251	1

The occurrence here of two red ♀'s and one white male may be accounted for, if, in the F_1 ♂ (X^wY^r), an interchange occurred about once out of 451 times, as shown in Fig. 131. Similar results were obtained when white and brown males were crossed, but no cross-overs were recorded. When variegated red females and white males were crossed, the same kind of results were obtained with 11 cross-overs out of 172 individuals in the back-cross.

FIG. 131.

Diagram illustrating crossing-over between genes for red and for white carried by the X- and "Y-chromosome" of an F_1 male fish. These genes are interpreted as allelomorphs.

Winge (1922-1923) extended Schmidt's experiments with Lebistes, and independently reached the same conclusions concerning the Y-chromosomes as had Aida. The results of a cross between a female of one race, X_oX_o, and a male of another race, X_eY_m, are shown in Fig. 128. The ripe germ-cells of this heterozygous male are represented by two non-cross-over classes, X_e and Y_m, and two cross-over classes, X_o and Y_{em}. Correspondingly there were two

kinds of males, $X_o Y_m$ and $X_o Y_{em}$. The latter are rare, one out of 73 sons.[2] Whether crossing-over also occurs in the female cannot be determined from Winge's data, since he gives no cases of $X_e X_m$ females. Moreover, he represents one type of female as X_o and implies that the X_o-chromosome is lacking in certain genes. Two pairs of genes are necessary to show crossing-over when two X's are present. In fact, Winge represents an X_e that has crossed over to a Y_m as X_o without representing the reciprocal allelo-
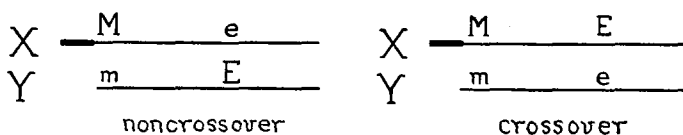
Fig. 132.

Diagram illustrating the possible relations of an attached X-chromosome to crossing-over between the autosomal portion of this compound chromosome and the autosome (the male of the latter) here called Y.

morphic change. The full formula should represent one of the X's containing the genes M and e, and the Y, in this case, as containing the genes m and E. After crossing-over the X would then contain E and M and the Y would contain e and m, as shown in Fig. 132. The X-chromosome after crossing-over is not X_o, but X_{ME}, and the Y-chromosome Y_{me}. If m and e are dominant over M and E, the results would be as recorded, except that another cross-over is expected, namely, X_{ME}. If the part of the X to the left of the M contains the sex-determining genes (the heavier part of the X in the figure) the absence of this cross-over in the experiment might be explained as due to the proximity of M to the X-component.

In a later paper Winge (1927) has reported nine genes

[2] In another experiment 4 cross-overs out of 68 sons are recorded.

in the Y- and three in the X-chromosomes of Lebistes, that have, as yet, shown no crossing-over. He suggests that this is either because they are close to the male-determining gene in the Y-chromosome, or else that they are identical with the male-determining gene. Five other genes show crossing-over between the X and Y, and one gene is autosomal. He represents the male-determining gene as single and dominant and leaves open the question of the nature of the allelomorph in the X-chromosome, labeling it zero.

Degeneration of Male-Producing Sperm.

In two closely related families of bugs, the Phylloxerans and Aphids, belonging to the XX-XO type, the male-producing class of sperms (no X) degenerate (Fig. 133). This leaves only the female-producing sperms (X). The sexual egg (XX), after extrusion of two polar bodies, is left with one X-chromosome. Fertilized by the X-sperm, these eggs produce only females (XX). These females are called stem mothers. They are parthenogenetic and become the starting point of a succession of other parthenogenetic females. After a time, some of these females may produce male offspring, others producing sexual females. The latter are diploid, like their mothers, but in them the chromosomes conjugate and their number becomes reduced to half. The former individuals that produce males do so by a process that will be described in the next section.

The Elimination of One X-Chromosome from a Diploid Egg to Produce a Male.

In the Phylloxerans, as stated above, a certain kind of female appears near the end of the parthenogenetic cycle whose eggs are a little smaller than those of the earlier females. Just before maturation of the smaller eggs the

X-chromosomes come together (there are four X's present). Two of them pass out of the egg into the single polar body that is given off (Fig. 134). The autosomes at this time divide, and half of each is eliminated. The
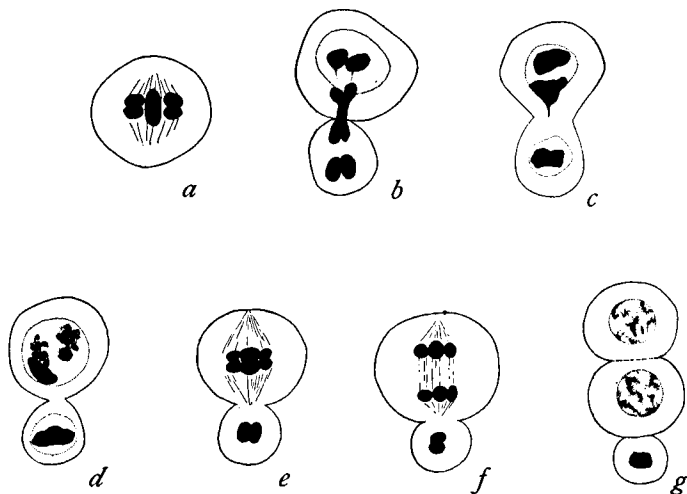

FIG. 133.

First maturation division of the bearberry aphid. At the first division, *a-c*, the large X-chromosome passes into one cell. At the second division, *e, f, g,* this cell divides again producing two functional female-determining sperms. The rudimentary cell does not divide again.

egg is left with a diploid set of autosomes and half of the X-chromosomes. It develops, by parthenogenesis, into a male.

In the Aphids a similar process takes place. The actual extrusion of one of the X's from the egg (there are only two X's present) has not been observed, but since, after the single polar body is given off, there is one less chromosome present in the egg, there can be no doubt but that one is lost, as in the Phylloxerans.

In these two groups the male sex is determined by a

different process from that which takes place in other insects, but the same mechanism is utilized in a different way to bring about the same end-result.

There is one further fact of unusual interest in this case. In the Phylloxerans the female that gives rise to the male eggs—she is called the male-egg producer—forms smaller eggs than did her parthenogenetic forbears. The⁻
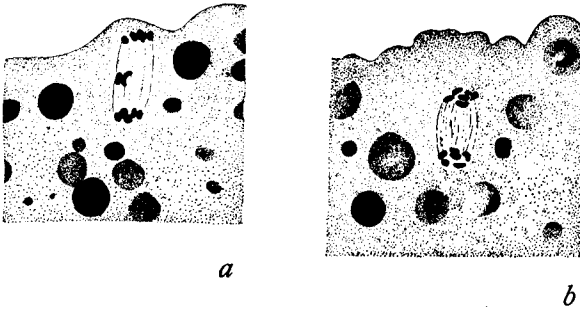

a

b

Fig. 134.

a, The first polar spindle of a ''male egg'' of Phylloxera in which two chromosomes lag on the spindle and are ultimately thrown out of the egg, leaving five chromosomes in the egg nucleus. b, The first polar spindle of a female egg, in which all six chromosomes divide leaving six chromosomes in the egg nucleus.

fate of the eggs is, therefore, indicated before the X-chromosomes are eliminated from them. It might appear that, here, sex is determined by the size of the egg, which might mean the amount of cytoplasm contained in it; but the conclusion is an illegitimate inference from the evidence, since the egg becomes a male only after half of its X-chromosomes are eliminated. What would happen if they were retained we do not know—probably the egg would develop into a female. At any rate, we have here an instance of a change that has taken place in the mother that leads to the formation of the smaller egg, which, in turn, reduces the number of its X-chromosomes to pro-

duce a male. The nature of the change in the mother is
unknown at present.[3]

*Sex-Determination through the Accidental Loss
of a Chromosome in Spermatogenesis.*

In hermaphroditic animals no sex-determining mecha-
nism has been found, and none is expected, since all the
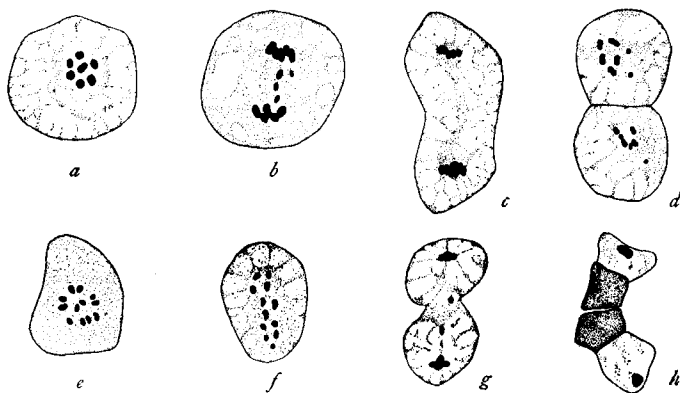individuals are alike, each with an ovary and a testis. In

FIG. 135.

First and second maturation division of the sperm-cell of Angio-
stomum nigrovenosum. At the second division (lower line) one of
the X-chromosomes gets caught in the division-plane. (After
Schleip.)

one species of nematode worms, Angiostomum nigro-
venosum, there is an hermaphroditic generation that alter-
nates with a generation consisting of males and females.
Boveri and Schleip have shown that when the sperm-cells
mature in the parthenogenetic generation (Fig. 135) one

[3] In one of the worms, Dinophilus apatris, eggs of two sizes are produced
by each female. Both kinds give off two polar bodies, resulting in a haploid
pro-nucleus. Both kinds of eggs are fertilized; the larger egg produces
females, the smaller one males (Nachtsheim). At present the cause of the
production of two kinds of eggs in the ovary is entirely unknown.

of the X-chromosomes frequently gets lost (being caught in the division plane) and this leads to the production of two classes of sperm, with five and six chromosomes. In the maturation of the eggs of the same female the
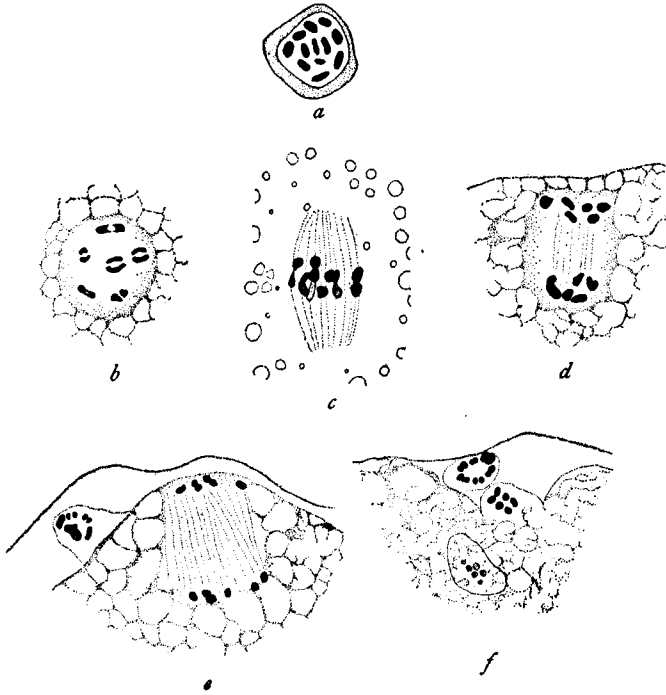

FIG. 136.

The two maturation divisions of the egg of Angiostomum nigro-venosum. Six chromosomes are left in the egg nucleus. (After Schleip.)

twelve chromosomes conjugate, giving six gemini (Fig. 136). At the first maturation six go into the first polar body and six remain in the egg. These split and six daughter chromosomes go into the second polar body, leaving six chromosomes in the egg, each with one X-chromosome. An egg fertilized by a sperm with six chromosomes

produces a female; an egg fertilized by a sperm with five chromosomes produces a male. Here an accident in cell-division becomes the mechanism of sex-determination.

Diploid Females and Haploid Males.

In the rotifers there is, first, a long series of generations of parthenogenetic females with the diploid number of chromosomes. No reduction takes place in the eggs and one polar body is given off. The series may apparently continue indefinitely under certain conditions of nourishment. The series can, however, as shown by Whitney, be brought to an end by a change in diet—such as feeding the females on a green flagellate. A female feeding on such a diet now produces daughters (by parthenogenesis) with dual possibilities. If one of these daughters is fertilized by a male (that may have then appeared), each egg, before maturation, is entered by a single sperm. The egg enlarges in the ovary and a thicker shell is laid over it (Fig. 137). It gives off two polar bodies, and then the sperm nucleus (haploid) unites with the haploid nucleus of the egg, restoring the full number of chromosomes. This egg is a resting or winter egg. It contains the diploid set of chromosomes, and after a time develops into the stem mother of a new line of parthenogenetic females, etc.

On the other hand, if the female in question is not fertilized, she produces eggs that are smaller than the ordinary parthenogenetic eggs. The chromosomes conjugate, and two polar bodies are given off. The egg is left with a haploid set of chromosomes. It segments, without doubling the number of its chromosomes, and produces a male. It is not clear what happens during the formation of the spermatozoa in the haploid males. Neither Whitney's (1918) nor Tauson's (1927) work gives a convincing account of the changes that take place.
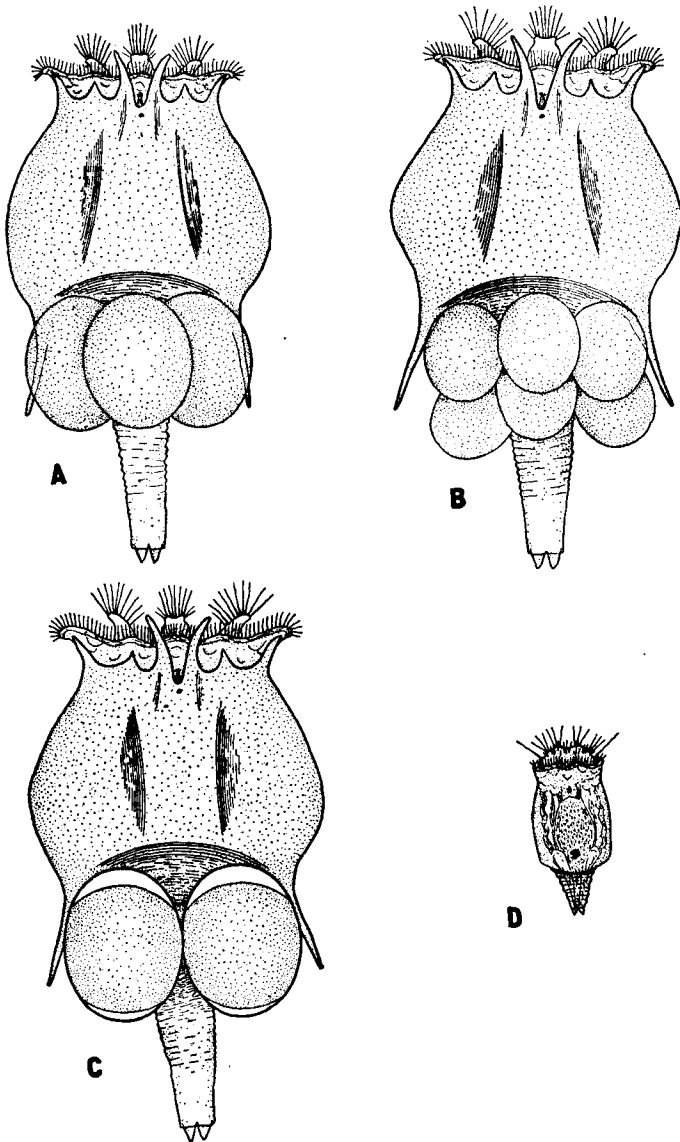
FIG. 137.

The rotifer, Brachionus bakeri. A, female with attached partheno-
genetic female-producing eggs. B, female with attached partheno-
genetic male-producing eggs. C, female with attached sexual eggs.
D, male. (After Whitney.)

The evidence, taken at its face value, appears to mean that the haploid number of chromosomes produces a male, the diploid a female. The presence of sex-chromosomes is nowhere apparent, hence the presence of specific sex genes cannot be assumed. Even if the absence of such genes be granted, it is not apparent why the half number of chromosomes should produce a male and the diploid number a female, unless the differential factor here involved be the relation between the amount of cytoplasm in the two kinds of eggs and the number of chromosomes present. Even then, however, the result is difficult to bring into accord with the case of the bee (described below), where the diploid egg, that produces a female, and the haploid egg, that produces a male, have the same size. The outstanding fact in both cases is that the haploid number of chromosomes is correlated with the male sex, even although something else determines in the rotifer which eggs become haploid.

It might be possible to invent an explanation involving sex-chromosomes if two kinds of X-chromosomes were postulated and if, at the reduction division, one passes out into the polar body of the male egg and the other one from the sexual egg (both being retained in the parthenogenetic egg); but it must be confessed that at present there is no excuse or need perhaps for advancing such a speculation.

Sex-determination in bees, and in their near relatives, the wasps and ants, is also connected with the diploid and haploid condition of the nuclei. The facts seem well established, but the interpretation here is also obscure. The queen bee deposits eggs in the queen-cells, in the worker-cells, and in the drone-cells. These eggs are, before being laid, all alike. The eggs in the worker-cells and the queen-cells are fertilized at the time of deposition; in the drone cells the eggs are not fertilized. All eggs give off two

polar bodies. The egg nucleus is left with the haploid number of chromosomes. In the fertilized eggs the sperm brings in a haploid set of chromosomes, which, uniting with the egg nucleus, gives the diploid number. From these eggs females develop (queens or workers). The queens owe their more complete development to the food supplied to the larvae in the queen-cells. This food is different from that given to the larvae in the worker-cells. The males (drones) are, as has been said, haploid.[4]

In this case, the determination of sex cannot be supposed to be due to any effect preceding maturation. There is no evidence that the presence of the sperm-nucleus in the egg affects the way in which the maturation division of the chromosomes takes place. Furthermore, there is no evidence that the environment (drone-cell or worker-cell) has any effect on the course of development. There is, in fact, no evidence here that any particular set of chromosomes has been set apart as sex-chromosomes. The only known difference between the two kinds of individuals, females and males, is the number of chromosomes present. We can, at present, only fall back on this relation as the one that is in some unknown way co lated with sex-determination. At present it cannot be satisfactorily brought into line with other cases in insects, where sex is related to a balance between genes in the chromosomes, but it may still be due to a balance between the chromosomes (genes) and the cytoplasm.

There is one further fact that involves sex-determina-

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[4] It is known that, as the cleavage of the unfertilized egg of the male proceeds, each chromosome breaks into two parts (except possibly in the nuclei that pass into the germ-track). This process does not appear to be a ''division'' of each chromosome, but rather its breaking or separating into two pieces. If this interpretation is correct there is no actual increase in the number of the genes and the occurrence of this process (also known in some of the nematodes) does not throw any light on the question of sex-determination.

tion in bees. When the maturation of the germ-cells in the male takes place, the first division is abortive. A small cell is pinched off without chromosomes (Fig. 86). At the second division the chromosomes divide. Half pass into one cell, that is very small and later degenerates; half remain in the larger cell, that becomes the functional spermatozoön and contains the haploid number of chromosomes. This number it brings into the egg, which, as stated, then develops into a female.

There are a few cases on record (Newell) where two races of bees have been crossed and the progeny of the hybrid recorded. The males are said to show the characters of one or the other original race. This is expected, in so far as the two races differ in genes in one and the same pair of chromosomes, because these would be separated at reduction, and one or the other would be retained in the haploid egg that produces a male. But if the racial differences depend on genes lying in different pairs of chromosomes, no such sharp distinction into two classes of grandsons is to be expected.

The worker bees (and ants) occasionally lay eggs. These become males, as a rule, which is expected, since the workers cannot be inseminated by the drones. There are records in ants of the rare appearance of sexual females from workers' eggs. It may be supposed that this is due to the retention of a double set of chromosomes. In the ''Cape bees'' the production of females (queens) from workers' eggs is said to be a common occurrence. Provisionally we may apply the same explanation as that given above for the females of worker ants that rarely produce eggs some of which, under special conditions, develop into females.

The direct transmission of the characters of the mother to her haploid sons has been more completely demonstrated in Whitings' work on the parasitic wasp, Habro-

bracon. The common type has black eyes. A mutant male with orange eyes appeared in the cultures. Crossed to black-eyed females, there were produced by parthenogenesis 415 black-eyed sons, and from fertilized eggs 383 black-eyed daughters.

Four of these (F_1) daughters, when isolated, produced parthenogenetically 268 black-eyed males and 326 orange-eyed males and no females.

Eight other F_1 daughters (from the original orange male) were mated with their F_1 brothers. There were produced 257 black-eyed sons, 239 orange-eyed sons, and 425 black-eyed daughters.

The original mutant orange-eyed male, when bred to his F_1 daughter, gave 221 black males, 243 orange males, 44 black females, and 59 orange females.

These results are expected on the hypothesis that the male is haploid and comes from an unfertilized egg. The gene for orange eyes and that for black eyes separate in the germ-cells of the hybrid mother when her germ-cells mature, half of the gametes then have one kind of gene, half the other kind. Any pair of genes in any pair of chromosomes will give the same result.

The reciprocal cross was also made, namely, an orange female was crossed to a black male. Eleven such matings gave 183 black daughters and 445 orange males, as expected; but twenty-two matings gave, in addition to 816 black females and 889 orange males, *57 black males*. The occurrence of these black males calls for a different explanation. They have obviously come from eggs fertilized by a black-producing sperm. A possible explanation would seem to be that the haploid sperm-nucleus has developed in the egg, and has given rise to those parts from which the eyes at least have come. The rest of the egg might then get its nuclei from the haploid egg-nucleus. There is, in fact, some evidence that this is the correct

explanation, since Whiting has shown that some of these exceptional black males may breed as though all their sperm carried only the orange gene of the mother. But there are other facts indicating that in these cases the explanation is not so simple as this, for most of the black males are sterile, as well as the few daughters arising from those males that are fertile (the mosaic males).[5] Whatever the final solution may be for these exceptional cases, the main results of the crosses confirm the theory that the males are haploid.

Sex in Haploids.

The demonstration by Allen in 1919, that the cells of the female haploid gametophyte of the liverwort, *Sphaerocarpus,* have a large X-chromosome and that the cells of the male haploid gametophyte have a corresponding small Y-chromosome, gives a reasonable explanation of the differences shown by their prothallia (gametophytes). Similarly the experimental demonstration by the Marchals, by Wettstein and others that from each spore-mother-cell of dioecious mosses two spores arise that develop into female protonemata (gametophytes), and two other spores that develop into male protonemata (gametophytes), falls into line with Allen's results on the allied liverworts. It is customary to speak of the two kinds of gametophytes as female and male respectively since one produces eggs the other sperm cells (antherozoids). The

[5] According to Anna R. Whiting (1925), ''the black-eyed patroclinous males show a higher percentage of morphological abnormalities than do males and females normally produced. The majority of patroclinous males tested have been sterile, some have bred as blacks and been partially fertile, while a few mosaics have produced orange-eyed daughters and have been fully fertile. The orange-eyed daughters of patroclinous males are normal in morphology and fertility. The black-eyed daughters of patroclinous males are few in number and show a large percentage of abnormalities and are almost completely sterile.'' The exceptional males in Hadrobracon may explain some of the anomalous cases that have been recorded in honey bees.

succeeding generation, the sporophyte (zygote) arising
from the fertilization of the egg by the sperm, is some-
times said to be sexless or asexual. It has however one
X- and one Y-chromosome.

Some unnecessary confusion has arisen between the
terminology used for the moss and liverwort and that
employed for dioecious flowering plants in which the terms
female and male apply to the sporophyte (diploid) gen-
eration and not to the egg-cell (which is part of the haploid
generation within the embryo sac) and to the pollen grain
(which is also part of the haploid generation). It may
seem at first sight, that male and female are used in these
two groups in a different sense. There is no real contradic-
tion, however, except a verbal one arising from phyloge-
netic implications. If the two cases in question are stated
in terms of genes, the imagined difficulties disappear. In
the liverworts, for instance, the balance of genes in the
haploid gametophyte, containing the large X-chromosome,
leads to the production of egg-cells and the balance of
genes in the haploid gametophyte containing the small
Y-chromosome leads to the production of sperm-cells. The
egg-bearer is here called female, the sperm-bearer male.
In the diploid generation of those flowering plants that
are dioecious, where a differential pair of chromosomes
is present in the male, the balance of the genes in the
diploid generation between the autosomes and two X's
gives a female (an individual that produces eggs), and the
balance of the genes in the diploid generation between the
autosomes and the XY pair gives a male (an individual
that produces sperm-cells). Both in the liverwort and the
flowering plant the situation turns on a balance between
sets of genes. It may be that the same sets of genes are not
involved in both cases, or that some of them are the same,
others are different. The essential point is that in both
cases, differences in balance lead to two kinds of indi-

viduals which are called females and males since they produce eggs and sperm-cells respectively.

In criticism of these statements it may possibly be said that the facts have only been restated and not explained. This is quite true. All that has been attempted is to point out that the facts may be restated in such a way that there is no apparent contradiction involved in the two cases. We may look forward perhaps to the time when the number and nature of the genes involved in cases where a difference of balance produces two kinds of individuals, will be determined. Meanwhile there is nothing in the situation that need cause anxiety, and certainly nothing that can be used as a refutation of recent advances in an understanding of sex-determination.

The haploid condition in animals is characteristic of the gametes. There are no cases of alternating haploid and diploid generations as in plants. But there are two or three types at least in which one sex is diploid and the other haploid. In the Hymenoptera and a few other insects the female is diploid and the male haploid, at least in the early stages of development. In Rotifera the females are diploid and the males are haploid. There is no evidence in either group of sex-chromosomes as such. At present no explanation resting on experimental evidence can be offered to explain these relations. Until such evidence is forth-coming the possible theoretical interpretations that have been suggested are not illuminating.

In *Drosophila* on the other hand where a known sex mechanism is at hand, and where there is experimental evidence bearing on the problem of balance of the genes involved in sex-determination, there is a recent observation by Bridges that is significant. Two mosaic individuals were found which, from genetic evidence, seemed probably to be composites, in part haploid and in part diploid. In one case the haploid region includes the location of a sec-

ondary sex-organ, the sex-comb (present in the normal
male, absent in the female). In the mosaic the sex-comb is
absent in the region that is haploid. In other words the
haploid group of chromosomes consisting of three auto-
somes and one X gives, as anticipated, the same kind of
result as six autosomes and two X's. The balance is the
same in each although the haploid region of the mosaic
has only one X, as has the normal male; but in the male
this is offset by six autosomes.

The converse case is reported by Wettstein who pro-
duced by artificial means gametophyte moss plants that
were diploid. If these arise from a haploid female game-
tophyte cell they are female, and if from a haploid male
gametophyte cell they are male. In both cases the balance
remains as before. It is apparent that sex-determination
is not in these cases regulated by the number of chromo-
somes but by the relation of contrasted sets of genes or
chromosomes to one another.

Sex and its Definition in Lower Plants.

The question of sex terminology appears in its most
acute form in the results of recent work on certain groups
of gilled fungi or toadstools, Basidiomycetes. In this
group, according to the recent statement by Hanna "The
problem of sex has occupied the attention of mycologists
for more than a hundred years." The discoveries of Miss
M. Bensaude (1918), of Kniep (1919-23), of Miss Mounce
(1921-22), Buller (1924) and Hanna (1925) have brought
to light an extraordinarily interesting situation. In order
to simplify the description of this work, the recent paper
of Hanna is here followed closely. By means of a new and
refined technique, single spores from the gills of the toad-
stool can be isolated. From each spore a single mycelium
of hyphae can be grown in dung-agar cultures. Each such
monosporous mycelium can then be tested by bringing it

in contact with the others one by one. Certain of these combinations will unite and form a secondary mycelium on which "clamp connections" develop, indicating that the mycelia are of "opposite sexes." Later, fruiting bodies or toadstools develop from such mycelia. On the other hand, other combinations, when made, do not form secondary mycelia with clamp connections, and, as a rule, do not produce fruit bodies. The union is interpreted to mean that the mycelia in question are of the same sex.

		51	52	54	55	57	58	59	50	56	53
AB	51	−	−	−	+	+	+	+	−	−	−
	52	−	−	−	+	+	+	+	−	−	−
	54	−	−	−	+	+	+	+	−	−	−
ab	55	+	+	+	−	−	−	−	−	−	−
	57	+	+	+	−	−	−	−	−	−	−
	58	+	+	+	−	−	−	−	−	−	−
	59	+	+	+	−	−	−	−	−	−	−
Ab	50	−	−	−	−	−	−	−	−	−	+
	56	−	−	−	−	−	−	−	−	−	+
aB	53	−	−	−	−	−	−	−	+	+	−

TABLE 1.

Now, when monosporous mycelia from the same strain (*i.e.,* from plants living in the same locality) are tested, the results are like those in Table 1. Here, the presence of clamp connections, formed after the union of two monosporous mycelia, is indicated by the + sign. Failure in this respect is indicated by the − sign. In the Table the mycelia fall into four groups (those belonging to the same group have been brought together in the Table). The result is interpreted as meaning that the spores of a single fruit body of the species studied, namely, Coprinus lagopus, belong to four sexual groups.

These four groups as Kniep first showed, can be accounted for on the assumption of two pairs of Mendelian factors that may be designated as Aa and Bb. If these factors are segregated, at the time when the spores are formed on each basidium, there will be present on each toadstool four kinds of spores, AB, ab, Ab, aB, each of which gives rise to a mycelium of the same genetic composition. As shown in Table 1, only those mycelia differing in both factors unite and form clamp connections. This means that there are four sexes, and only those with different sex factors can combine.

There is also a cytological background that accords excellently with these genetic assumptions. The nuclei in the monosporous mycelia lie singly in the protoplasm. After the union of two mycelia, the nuclei in the derived (secondary) mycelium are in pairs. It is reasonable to assume that one member of each pair has come from one mycelium, the other one from the other mycelium. At the time when the four spores are about to develop, reduction is supposed to take place, so that each spore comes to contain a reduced nucleus. Each spore gives rise to a new reduced mycelium. Such a picture accords with the reduction process in higher plants and animals and serves to bring these moulds into line with the genetic results that take place when the chromosomes are reduced from diploid to haploid gametes. It is true that, as yet, it has not been shown in *Coprinus* and related species that we are dealing with a diploid-haploid relation, but it seems not improbable that this is a correct interpretation of the facts as far as known. If so, the sorting out of the genetic factors in these toadstools is the same in principle as that shown by other plants and animals.

The preceding relations hold for strains occurring in any one locality. If strains from different localities are tested, an extraordinary result is found. All the monos-

porous mycelia of one strain unite (*i.e.*, give mycelia with clamp connections, etc.) with all the monosporous mycelia of the other strains. In Table 2 the pairing of eleven

		A^4B^4			a^4b^4		A^4b^4			a^4B^4	
		4	7	8	5	2	6	10	11	1	3	9
A^2B^2	25	+	+	+	+	+	+	+	+	+	+	+
	26	+	+	+	+	+	+	+	+	+	+	+
	27	+	+	+	+	+	+	+	+	+	+	+
	28	+	+	+	+	+	+	+	+	+	+	+
a^2b^2	20	+	+	+	+	+	+	+	+	+	+	+
	23	+	+	+	+	+	+	+	+	+	+	+
	24	+	+	+	+	+	+	+	+	+	+	+
a^2B^2	21	+	+	+	+	+	+	+	+	+	+	+
	29	+	+	+	+	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+	+	+	+	+
A^2b^2	16	+	+	+	+	+	+	+	+	+	+	+

TABLE 2.

monosporous mycelia from a fruit body from one locality (Edmonton, Canada) are shown to have united with eleven monosporous mycelia from another locality (Winnipeg). The same results were obtained whenever strains from different localities were paired. The combinations carried out by Hanna gave twenty sexes for *Coprinus* and no doubt the number could be greatly increased by extending the combinations to other localities.

Not only was this cross-hybridizing carried out, but the factorial hypothesis was further tested by experiments on the crossed strains themselves. If the factors from the different strains are treated as allelomorphic pairs, and the factors in one strain are designated by Aa and Bb, and those in another strain by A^2a^2 and B^2b^2, there will be sixteen possible hybrids formed by the union of the

mycelia from these two varieties, and each mycelium of such hybrid origin will behave in a manner similar to that shown by mycelia from a single source, in that two given mycelia form clamp connections only in case they carry no factors in common.

Here we have the phenomenon of sex exhibited on a grand scale if we interpret the factors involved as sex factors in the conventional sense. There is no objection to using the word in this way if it seems advantageous to define sex on such a basis. Personally, I think it is simpler to interpret the results in the same way that East has interpreted the results of his tobacco investigations, and call the factors involved self-sterility factors (see below). The explanation is the same in principle, whatever language one prefers to use in describing them.

Under the title of Research on Relative Sexuality, Hartmann has recently described results that he obtained with the marine alga, *Ectocarpus siliculosus*. The motile swarm-spores set free from the plants are externally alike, but according to their subsequent behavior they fall into two classes ''females'' and ''males.'' The former
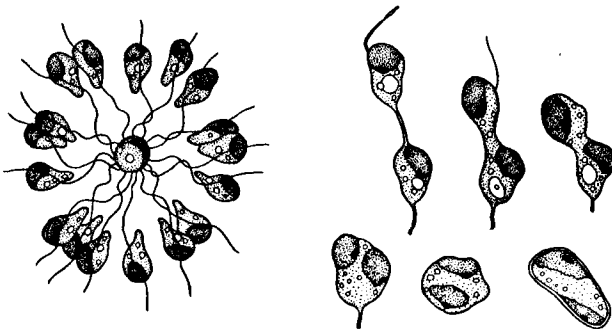

FIG. 137a.

Swarming of male gametes around a ''female'' gamete (to left). Union of ''male'' gamete and a female gamete (to right). (After Hartmann.)

quickly settle down, while the latter continue to swarm for some time and surround the female individual (Fig. 137a). One of the male swarm-spores fuses with the resting female swarm-spore. Hartmann isolated individual parent plants and when the swarm-spores were set free, tested these *en masse* against each other. A typical result is given in Table 3 (to the left) where union is indicated by

	3♂	4♀	5♀	7♀	11♂	13♀	14♀
3♂	−	+	+	+	−	+	+
4♀	+	−	−	−	+	+	−
5♀	+	−	−	−	+	−	−
7♀	+	−	−	−	−	+	−
11♂	−	+	+	−	−	+	−
13♀	+	+	−	+	+	−	+
14♀	+	−	−	−	−	+	−

	31♀	32♀	33♂	35♂	38♂	40♂
31♀	−	−	+	+	+	+
32♀	−	−	+	+	+	+
33♂	+	+	−	+	−	−
35♂	+	+	+	−	+	−
38♂	+	+	−	+	−	−
40♂	+	+	−	−	−	−

TABLE 3.

the + sign and failure to unite by the − sign. Each kind is tested against each of the other kinds. In most cases the swarm-spores from a given individual behave constantly either as males or as females with the other types, but in a few instances swarm-spores that acted as females in some combinations acted as males in other combinations. Thus No. 4 (see Table 3, to left) and No. 13 give results inconsistent with their reaction in other combinations. Another exception is found in the reactions between No. 35 and No. 38 (see Table 3, to right), both of which are designated as males from their other behavior, yet react to each other as male and female. Hartmann speaks of certain individuals as strong females and others as weak females according to the number of "clusters" that they give in different combinations, and concludes that weak females may function as males when opposed to

strong females, and that weak females may function as females when opposed to strong males. To what extent these relations may be affected by age-factors (settling down, for example), or by environmental factors is not entirely clear, although Hartmann's statement that the relations hold from day to day as the swarm-spores are tested seems to exclude such explanations. Unfortunately the material is not suited to a genetic analysis of the factors involved. Whether the quicker settling down of the gametes from a certain individual is a sufficient index of their "sex" and if so, how this is changed when a weak female acts as a male, etc., is not apparent. Nevertheless the failure of gametes from the same plant to copulate appears to put the phenomenon in the same category as self-sterility and its correlative cross-fertility. To designate this as a criterion of sex is perhaps largely a matter of choice or of definition at present. Personally, it seems to me to confuse rather than to clear up the problems involved to use the word sex in this connection where the phenomenon is one of union or non-union of gametes rather than one of sex as commonly understood.

The question then may be asked whether it may not be simpler and less likely to lead to confusion to call the factors, involved in the union of the mycelia of *Coprinus* and of the swarm-spores of *Ectocarpus,* self-sterility factors rather than sex-factors. The recent important results of East in his studies on self-sterility in tobacco have for the first time put the oft-studied problem of cross- and self-fertility in flowering plants on a well-tested genetic basis. These phenomena in the flowering plants have many resemblances to the union of the gametes in *Coprinus* and *Ectocarpus* and while the *modus operandi* of the process may not be identical in all cases, there is much to suggest that the genetic and physiological backgrounds may be essentially alike.

In a brief paper East and Mangelsdorf have given a summary of work extending over several years dealing with the inheritance of self-sterility in crosses between two species of tobacco, viz. *Nicotiana alata* and *N. forgetiana*. Only the most general conclusions can be given here. By special manipulation several selfed, homozygous strains of self-sterile individuals were carried through twelve generations to obtain suitable material for testing the problem. As an example the results of one type of the families produced may be given. Three kinds of individuals a, b and c were found. Each individual of any one of these kinds is sterile with any other individual of its kind and fertile with each individual of the other two kinds; but the progeny resulting from reciprocal crosses is different. Thus, a ♀ by c ♂ gives b and c individuals only, while c ♀ by a ♂ gives only a and b individuals. Two classes always appear in equal numbers, but the class of the mother is never represented in the offspring. The explanation is as follows. If three allelomorphic genes are present in such a family $S_1S_2S_3$ and if class a $= S_1S_3$; class b $= S_1S_2$; class c $= S_2S_3$ and if the pistil of a plant affords the stimulus for the growth of the pollen which bears sterility factors other than its own, the results find a consistent explanation. For instance, plant c (S_2S_3) affords a sufficient stimulus only to pollen carrying factors other than S_2S_3. Only pollen bearing the factor S_1 can penetrate the style and fertilize the eggs. The progeny will be S_1S_2 (class b) and S_1S_3 (class a) in equal numbers. Reciprocally, a ♀ (S_1S_3) by c ♂ (S_2S_3) permits the S_2 pollen alone to penetrate the eggs giving S_1S_2 (b) and S_2S_3 (c). This result which is typical of all the others, explains why the female combination is absent in the progeny, why the progeny of reciprocal crosses is different and why the progeny-counts of two classes other than female are equal regardless of which of the other two classes serves as the male parent.

There are several ways of testing the validity of this hypothesis. The tests have been made and the hypothesis confirmed. This convincing analysis, the result of carefully planned genetic experiments, is a contribution of the first rank to a problem that has baffled students of fertilization for seventy-five years and more. The solution is not only a keen genetic analysis of the case but gives an insight into the physiological reactions between the haploid pollen tube and the diploid tissue of the female. It has been shown by direct observation that the rate of growth of the pollen tube in the tissue of the female is consistent with the view that a differential rate of growth is actually present. The nature of the relation is not known at present but may reasonably be assumed to be chemical. It is possible that the same or a similar chemical reaction with its genetic basis may account for the self-sterility observed in the union of the genetically unlike mycelia of the lower forms. If this can be established the genetic problem has to do primarily with self-sterility factors that are probably Mendelian genes. It may seem of doubtful value to identify these factors with sex factors which conventionally at least apply to somatic differences in dioecious types or to those with separate sexes. It is true that amongst these differences are those concerned with producing eggs and sperm whose main function is to unite with each other, but, as generally understood, these functions are less conspicuous than those appertaining to the bodily constitution of male and female individuals.