CHAPTER III

THE MECHANISM OF SEGREGATION

ONE of the most secure generalizations of modern work on the cell is that every cell of the individual contains a *constant number* of self-perpetuating bodies (called chromosomes), half of which are traceable to the father and half to the mother of the individual. No matter how specialized cells may be, they contain the same number of chromosomes. Equally important is the fact that after the eggs of the female and the sperm-cells of the male have passed through the ripening or maturation divisions the number of chromosomes is reduced to half.[1] Lastly, there is convincing evidence that the reduced number of chromosomes is brought about as the result of a separation of such a kind that each mature germ-cell gets only a paternal or a maternal member of each chromosome pair.

The reduction takes place in the female at the time when the polar bodies are given off from the egg; and in the male just prior to the formation of the spermatozoa. A characteristic process is seen in the oögenesis and spermatogenesis of the nematode worm *Ancyracanthus cystidicola* (a parasite in the swim-bladder of fresh-water fishes) described by Mulsow. The young eggs contain twelve chromosomes (Fig. 14, *a*). As the result of the later union of these twelve in pairs, six short threads appear in the nucleus of the egg just before it extrudes its polar bodies. The threads contract to six short rods (split in two planes at right angles to each other), the tetrads (Fig. 14, *c*). With the dissolution of the nuclear wall these tetrads are set free in the protoplasm, and a spindle develops about them (Fig. 15, *a*). They pass to the equator of the spindle, and there dividing lengthwise,

[1] Exceptions occur in certain cases of parthenogenesis.

half of each goes to one pole, and half to the other pole of the spindle (Fig. 15, *b*). One end of the spindle protrudes from the egg, and around it the protoplasm con-

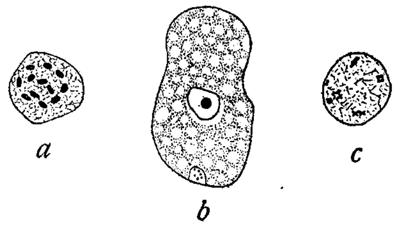

FIG. 14.—Oöcyte of *Ancyracanthus*, *a;* growth period, *b*; nucleus with tetrads, *c*. (After Mulsow.)

stricts off (Fig. 15, *c*) to form the first polar body. About the six ovoidal chromosomes left in the egg a new spindle develops; and these chromosomes become drawn into its equator, where they divide again, half of each going

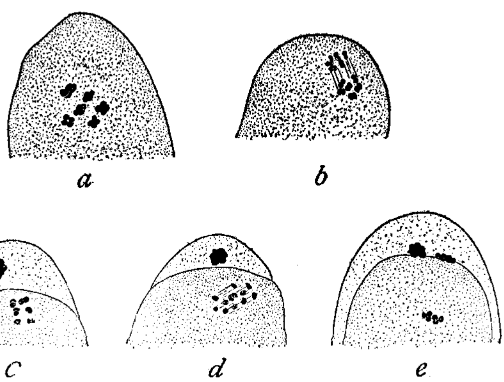

FIG. 15.—Egg of *Ancyracanthus* with six tetrads, *a;* egg with first polar spindle, *b;* egg after extrusion of first polar body, *c;* egg with second polar spindle, *d;* egg after the extrusion of both polar bodies, *e*. (After Mulsow.)

to one pole and half to the other (Fig. 15, *d*). A second protrusion takes place from the surface of the egg which pinches off to form the second polar body (Fig. 15, *e*). Thus, after two mitotic divisions, the egg has lost three-quarters of its chromatin, but retains half the full number of chromosomes, and as a result, the original twelve chromosomes have been reduced to six.

Around the six chromosomes left in the egg, a nuclear wall forms, and the chromosomes become spun out into delicate fibres. Meanwhile a spermatozoön has entered the egg, and out of its head another nucleus develops. The two nuclei, the egg nucleus and the sperm nucleus, move toward the center of the egg (Fig. 16, *a*), where they come into contact with each other. After a time, the chromatin threads begin to condense again into rods.

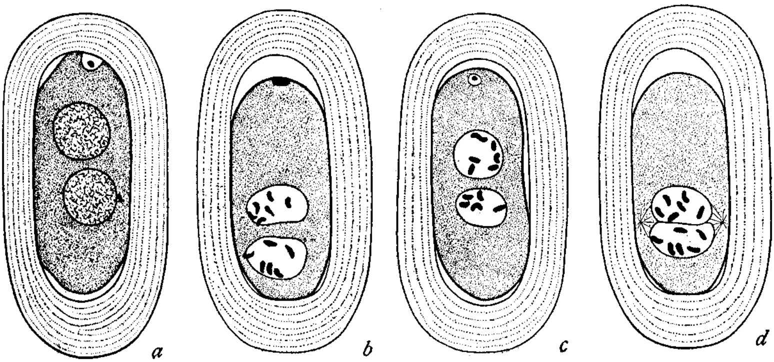

FIG 16.—Eggs of *Ancyracanthus* within membrane. Egg with two pronuclei, *a;* egg pronucleus with six chromosomes and sperm nucleus with six chromosomes, *b;* egg pronucleus with six chromosomes and sperm nucleus with five chromosomes, *c;* union of male and female pronuclei, *d.* (After Mulsow.)

Six appear in the egg nucleus, and six in the male nucleus (Fig. 16, *b*)[2]. A spindle develops in the protoplasm of the egg around the twelve chromosomes of which six have come from the father (the paternal chromosomes) and six from the mother (the maternal chromosomes) (Fig. 16, *d*). Each chromosome now splits lengthwise into equivalent halves, and a half moves to each pole of the mitotic spindle. The spindle rotates in the cytoplasm of this egg until its long axis corresponds with that of the egg. As the daughter chromosomes move towards the poles of the mitotic spindle the egg protoplasm constricts

[2] Assuming a female producing sperm to have entered.

between them so that two cells are formed, each cell containing twelve chromosomes, six paternal and six maternal. Thus, through fertilization, the whole number of chromosomes is restored to the egg. This number remains through all subsequent divisions of the cells of the embryo.

The male of *Ancyracanthus* has only eleven (Fig. 17, *a*) chromosomes; because the male has only one sex-chromo-

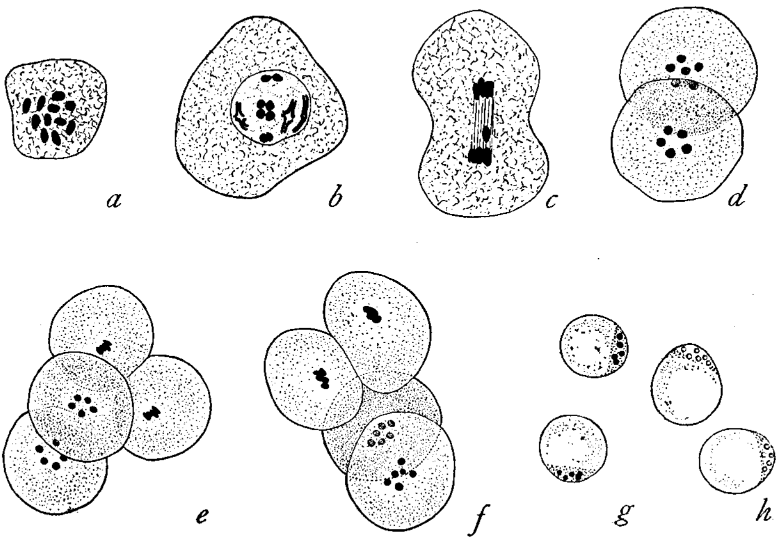

FIG. 17.—Spermatogenesis of *Ancyracanthus*. Spermatogonial cell, *a;* cell after growth period with tetrads, *b;* first spermatocyte division, *c;* two cells resulting from first division with six and with five chromosomes, respectively, *d;* four cells resulting from the next division, *e;* ditto, *f;* mature spermatozoa, one with six, the other with five, chromosomes, *g;* ditto, living spermatozoa, *h*. (After Mulsow.)

some, while the female has two sex-chromosomes. Both sexes have ten other chromosomes, sometimes called autosomes. Just before the maturation divisions take place, there are six rods in each sperm-cell, five of which (the autosomes) condense into tetrads, the sixth (the sex-chromosome) into only a double body (Fig. 17, *b*). A spindle develops about these and each of the five autosomes divides. The sex-chromosome does not divide, but passes to one pole of the spindle (Fig. 17, *c*). The result is that two cells are produced, one with six, the other with five chromosomes (Fig. 17, *d*).

Without a resting stage a new spindle develops in each cell, and a new division takes place—each dumb-bell-shaped body dividing, as well as the sex chromosome in the cell that contains it. In all, four cells result (Fig. 17, *e* and *f*)—two with five chromosomes each, two with six each. Each becomes a spermatozoön, which in this worm is a round cell with the chromosomes at one pole (Fig. 17, *g*). Half of the spermatozoa contain six, half five chromosomes. They can be distinguished even in the living sperms (Fig. 17, *h*). If a six-chromosome sperm fertilizes an egg (Fig. 16, *b*), a female (with 12 chromosomes) is produced—if a five-chromosome sperm fertilizes an egg (Fig. 16, *c*), a male (with 11 chromosomes) is produced.

The two chromosome divisions (or separations) that take place when the polar bodies are extruded from the egg are, for a number of reasons that need not be entered into here, generally regarded as equivalent to the two final divisions in the ripening of the sperm-cells. One of the two divisions is interpreted as an ordinary cell-division in which the chromosomes split lengthwise into equivalent halves—half going to each pole. The other division is interpreted as a separation of whole chromosomes that have come together side by side at an earlier stage. The tetrad is, then, looked upon as a pair of chromosomes that have conjugated in the sense that they have come to lie side by side (with interchange of materials at times in a way to be described later). One split is supposed to correspond to the line between the conjugated pairs; the other split represents a division in each chromosome of the pair. As a consequence when the chromosomes move apart (at the maturation division) one of the two divisions is said to be a "reducing division" because whole chromosomes are supposed to separate; the other division is said to be an "equation division," each chromosome splitting lengthwise into equivalent halves as in ordinary cell-division.

The interpretation of these two divisions that occur in the egg and in the sperm-cell has been the subject of much speculation. It is apparent that the process reduces the number of chromosomes by half, and that the whole number is regained by fertilization. It is sometimes said that the "purpose" of this division is to keep the number of chromosomes constant, for, if not reduced, they would increase in number with each fertilization.

The "reason" for the other, the second, division is acknowledged to be obscure. For present purposes it is futile to speculate concerning these two divisions, but it should be pointed out here that the genetic evidence is in full accord with the interpretation of these two divisions that is generally accepted to-day by cytologists, *i.e.*, that one of the divisions separates the conjugating pair, and that the other represents a longitudinal division within a paternal and within a maternal chromosome of each pair.

If we follow the history of the germ-cells further back before the maturation divisions, we find that between the stage when the half number of chromosomes reappears (tetrads) and the stage at which the full number was present, there is a very obscure period in the history of the germ-cells. This period has been studied chiefly in the male. Only a few types have been found favorable for the study of this period. One of the most favorable ones is a marine annelid, *Tomopteris,* studied by the Schreiners. The early division of the germ-cells (the spermatogonia) of *Tomopteris,* when the full number of chromosomes is present, is shown in Fig. 18, *a–g.* The division is like that of all the other cells of the body. The chromosomes appear as thick bent threads that split lengthwise (Fig. 18, *a, b*). The nuclear wall disappears and a spindle appears near the group of split chromosomes (Fig. 18, *c*). As the poles of the spindle move apart the chromosomes become arranged at the equator of the spindle, each half of each chromosome becoming attached by a spindle fibre to one pole (Fig. 18, *d*). The halves move

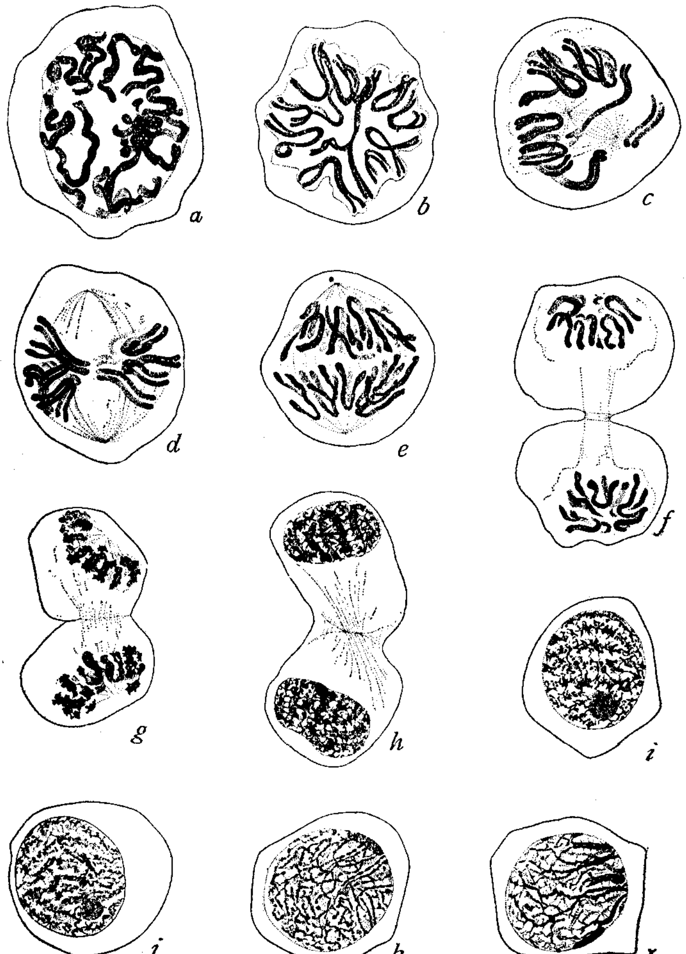

FIG. 18. Last spermatogonial division of *Tomopteris*, *a-h*; stages before and during synapsis, *i-l*. (After Schreiner.)

apart towards their respective poles (Fig. 18, *e*) and as they become separated into two groups the cell protoplasm constricts between them to produce new cells (Fig. 18, *f*). When the chromosomes have reached the pole they shorten (Fig. 18, *g*) and appear to send out anastomosing threads. Around this group of threads a new nuclear wall is formed (Fig. 18, *h*). All trace of the separate chromosomes is now lost, but between the last stage just described and the stage now to be described it is supposed that important changes in the chromosomes take place. This new phase is spoken of as the synapsis stage. At the beginning of this stage (Fig. 18, *i* and *j*) faint indications of the chromosome appear, and soon they can be seen again (Fig. 18, *k*) as long thin threads whose free ends place themselves in parallel pairs. The pairing of the threads continues to extend inwards from the ends (Fig. 18, *l*) until they have united throughout the length of the loops (Fig. 19, *a*). There are exactly half as many of these loops as there were original chromosomes, which is expected if they have united in pairs. The conjugation has been accomplished.

During the stages that follow, the double chromosomes shorten and become thicker (Fig. 19, *b, c, d*), and condense into the form of tetrads (Fig. 19, *e*). They begin to separate into halves, each half is also split lengthwise. A spindle appears, and the cells divide (Fig. 19, *f, g, h*). In each cell the chromosomes show indications of passing into a resting stage, as happens after all ordinary cell divisions, but before this change has gone very far a new spindle appears (Fig. 19, *i*), and preparations for another division are rapidly made. The new division completes the maturation of the sperm-cells (Fig. 19, *j, k, l*). Each of the four cells resulting from the original sperm-mother-cell differentiates into a spermatozoön.

In one of the salamanders, *Batrocoseps*, the maturation stages of the male are particularly well shown. The essential stages in synapsis are shown in Fig. 20, *a–d* as worked out by Janssens. These stages are essentially the same as those of *Tomopteris*. During the early multiplication stages the cells of the future testes divide by the ordinary mitotic process. The cells then pass into the synaptic stage (Fig. 20, *a–d*). As the chromosomes begin

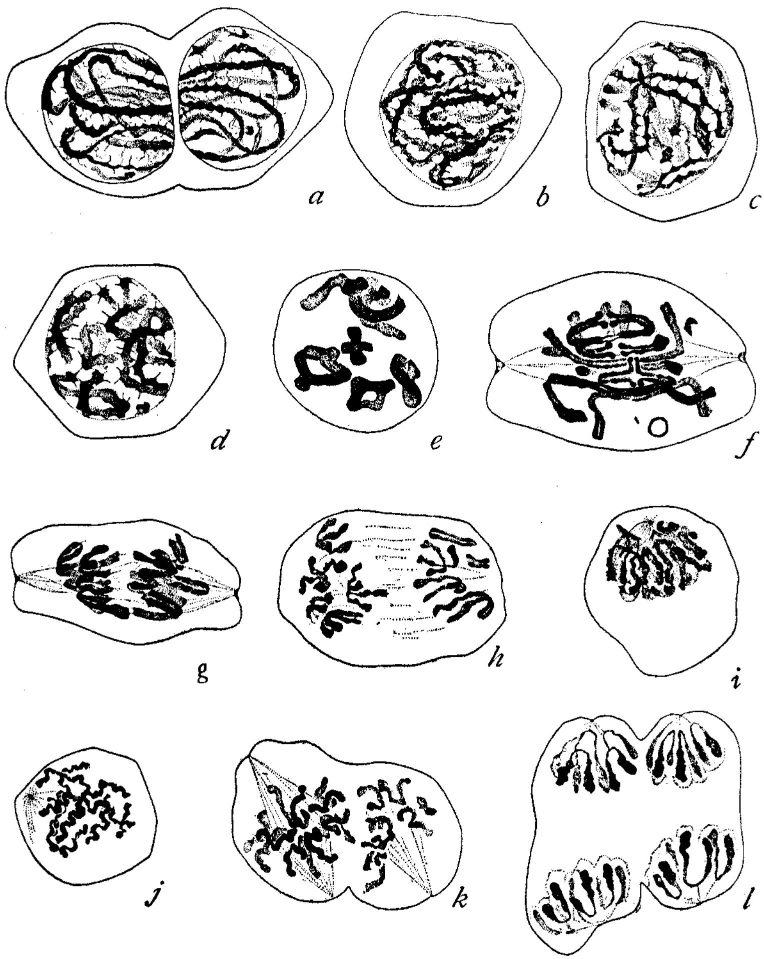

FIG. 19.—Thin-thread stage of *Tomopteris* spermatocyte, *a-d;* tetrads, *e;* first spermatocyte division, *f-i;* second spermatocyte division, *j-l.* (After Schreiner).

to emerge as thin threads, it is found in *Batracoseps* that their ends are all pointed towards one pole (Fig. 20, *d*). This is the same pole as that towards which the two ends of each V-shaped chromosome pointed as the cell went into the resting stage. It appears then that the chromosomes not only retain their original orientation, but that the ends of homologous chromosomes have already come

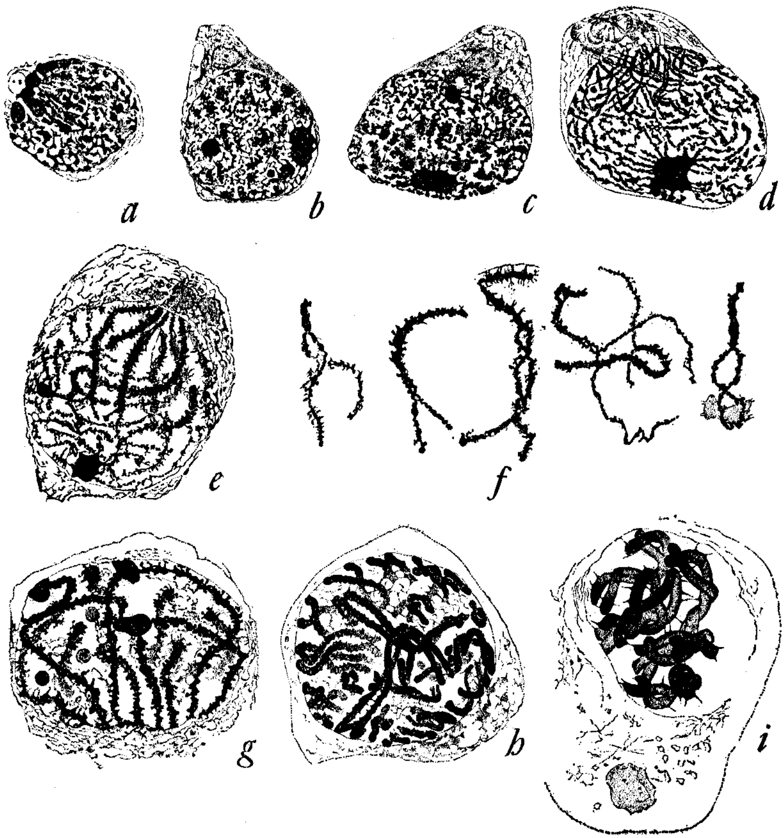

FIG. 20.—Synaptic stages and those immediately following in *Batracoseps*. (After Janssens.)

together, or are coming together, as the following stages show clearly.

The union that begins at the ends (Fig. 20, *e*) gradually extends along the length of the chromosomes, which are now in the form of thin threads. At the point where the two threads come together (Fig. 20, *f*) they can often be seen to be shaped like a Y and, at the point of meeting, the uniting threads are often twisted about each other.

The fused part of the united threads steadily grows shorter and thicker. They become the condensed pachytene threads, and appear as represented in Fig. 20, *g*. The thick threads shorten further, and the line of fusion between them (or a new line of cleavage) appears, as seen in Fig. 20, *h*. It will be noticed also that the ragged outline that the chromosomes had during the preceding stages is gradually lost, so that they now appear as solid rods or cords, which finally when they have reached the last stage in their condensation (Fig. 20, *i*) appear (in *Batracoseps*) as rods *twisted about each other*. Whether this twisting represents the original wrapping around each other of the leptotene threads as they conjugate, or whether it is a new arrangement resulting from the condensation of the chromosomes that are not free to move at all points, hence twist about each other as they condense, is a question that calls for further and careful consideration. For the present—since segregation alone is here involved—this matter may be laid aside. In this condensed condition the chromosomes pass into the first maturation division.

As already stated, the union of the chromosomes in the eggs of the female has been less often studied, but that the process is essentially the same is sufficiently evident. In one of the sharks, *Pristiurus melanostomus*, the following stages described by Maréchal show how similar are the maturation stages in the female to those in the male. When the germ-cells have reached the end of the multiplication period they pass into the synaptic condition, as shown in Fig. 21, *a* to *d*. Then threads appear in the nucleus; and soon it becomes evident that most of them are in the form of loops, whose ends are uniting in pairs (Fig. 21, *e*, *f*). When conjugation is finished thick loops are present that shorten further into thick rods (Fig. 21, *g*) that often show a single longitudinal split. The egg now begins to accumulate the enormous amount of yolk characteristic of selachian eggs; and during this time the chromosomes become more and more indistinct. As shown in the figure (Fig. 21, *h–k*) they appear to send out loops laterally, which loops may be only the bendings of a long thread. When the yolk formation is finished the chromosomes condense into shorter threads, with lateral branches (Fig. 21, *l*). When the egg is ripe, the nuclear wall is absorbed, the chromosomes appear as short rods *(arranged in twos), which place themselves in the polar* spindle. Two polar bodies are given off, leaving the reduced number of chromosomes in the egg.

It is obvious from the preceding account that the sperm and the egg pass through essentially the same stages during maturation, the essential feature of which is the conjugation of homologous chromosomes followed by their subsequent segregation. Each sperm and each egg is left with half the original number of chromosomes—one of each kind.

Lateral Versus End-to-End Fusion of the Chromosomes

In the preceding account of the union of the chromosomes only one method of union is described, viz., side-to-side conjugation. The tetrad as represented is due to one division plane between the conjugating pairs, and the other due to a longitudinal split of each conjugating member. But according to some observers, more especially botanists, another method of union also occurs, in which the split chromosomes unite end to end. If the division planes in such a tetrad represent respectively the plane of union at the ends, and the longitudinal split through the united rods, *the final result of this separation would be exactly* the same so far as the four elements of the tetrad are concerned, but the process would have serious conse-

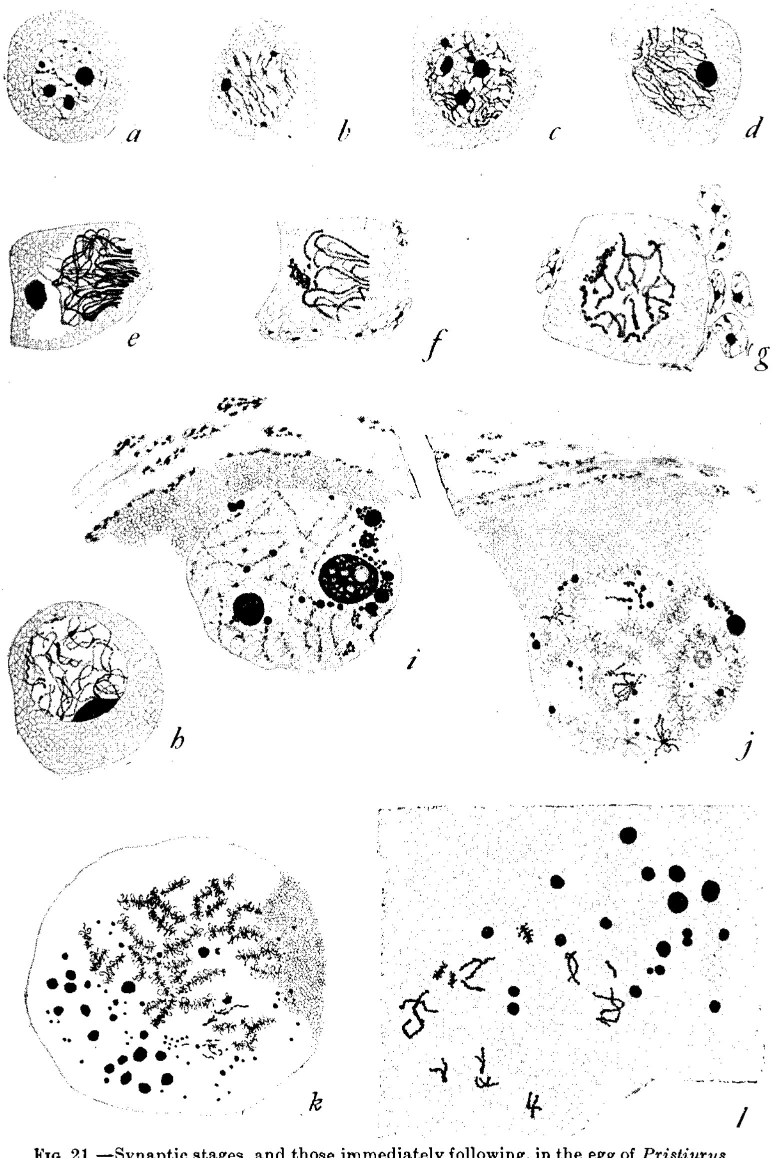

FIG. 21.—Synaptic stages, and those immediately following, in the egg of *Pristiurus*. (After Maréchal.)

quence for genetics in so far as the chromosomes represent the bearers of genes, for while side-to-side union offers an opportunity for interchange between the paternal and maternal members of a pair, no such interchange could be postulated if end-to-end conjugation took place. So far as segregation is concerned either method supplies all that is called for.[3] A discussion of other matters will be left until later.

Individuality of the Chromosomes

During the period of cell-division there can scarcely be any question concerning the persistence of the individual chromosomes, because they remain visibly distinct elements in the cell; but when the nucleus re-forms after each division the chromosomes spin out threads laterally, and these appear to fuse, making a continuous network throughout the nucleus. Whether there is actual fusion between these threads or whether they occupy delimited contact areas, and whether the branches represent the essential part of the chromosome concerned in heredity, are questions impossible to answer at present. The genetic evidence at least consistently shows that no real fusion of the hereditary material occurs even in cells that have passed through many such resting periods.

From several other sources there are strong indications that the chromosomes retain their individuality during the resting stage. In *Ascaris,* where the chromosomes are few and long, they are often drawn out in an irregular way in the cleavage cells as they pass to the poles of the spindle of the dividing cells. Daughter halves of the same chromosomes show the same identical irregularity. Boveri has shown by an examination of a large number of daughter cells (pairs) that are getting ready for the next division, that when the chromosomes of sister cells reap-

[3] If the pairs fused end to end and the tetrad arose by two longitudinal divisions, the outcome would not be in harmony with the theory of segregation based on separation of maternal and paternal chromosomes at reduction.

pear they show the identical irregularities (Fig. 22, *a* to *d*). It is probable, therefore, that each chromosome has retained the particular form that it had when it passed into the resting stage; or at least that the axial thread from which the network was spun out has remained in place.

In a few cases the chromosomes appear more or less visible during the resting stages. This, however, is such a rare event that it is doubtful whether it can be appealed to in support of the view that in other cases the chromosomes remain intact.

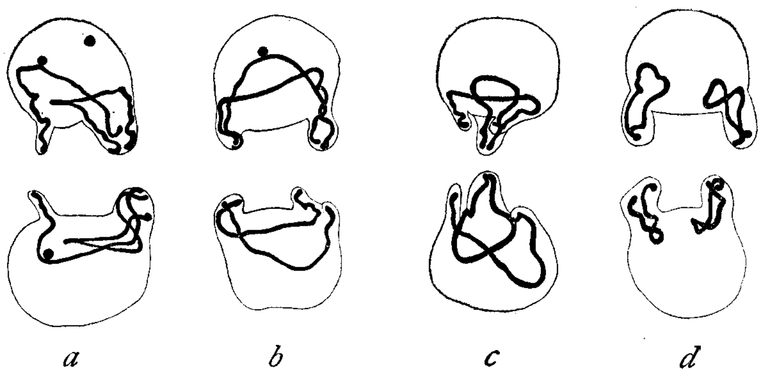

FIG. 22.—Sister blastomeres of *Ascaris* preparatory to another division, showing similar arrangements of chromosomes. (After Boveri.)

The most convincing evidence comes from exceptional cases of accidental or irregular distribution of one or more chromosomes, so that an egg, or a cell comes to have one more chromosome than is usually present. In the thread-worm *Ascaris* there are two varieties—one that has four chromosomes in the embryonic cells (with two as the reduced number) and another variety with two chromosomes (with one as the reduced number). A few females have been found in which the unfertilized eggs contain one of these numbers, and all of the spermatozoa that have been received from another individual the other number. In such cases the fertilized eggs, and all embryonic cells, have three chromosomes each (Fig. 64), showing that when an egg starts with three chromosomes, this number is retained through all subsequent divisions, despite the fact that after each division a resting stage intervenes.

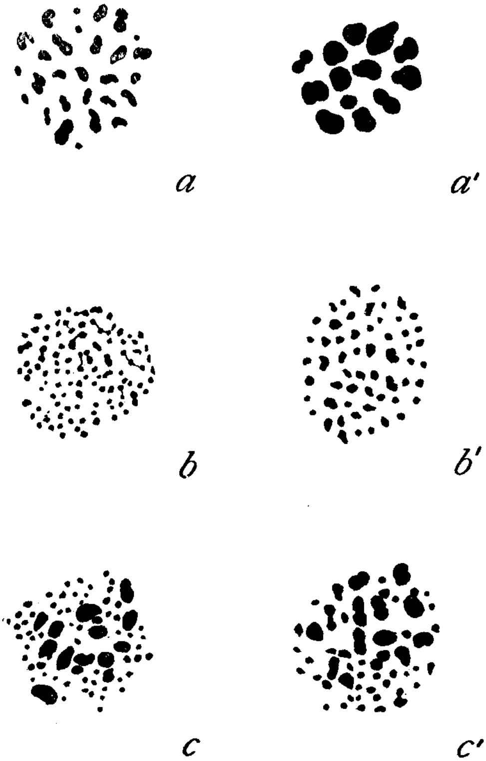

FIG. 23.—Normal a, b, and reduced a′, b′, chromosomes of two species of Biston; and of hybrid c, c′.

The evening primrose, *Œnothera Lamarckiana,* has 14 chromosomes (reduced number 7). Individuals are known in which there are 15 chromosomes. As a result of accidental displacement at a division in a germ-cell, possibly one cell came to contain an additional chromosome. Such a cell combining with a normal one, at fertilization, would produce a plant of the 15-chromosome type. Here again, the additional chromosome persists as an individual element of the cell throughout subsequent cell-generations.

FIG. 24.—Lagging and elimination of chromosomes in hybrid fish embryos. (After Pinney).

In *Drosophila* a female occasionally appears with two X's and a Y-chromosome. There are several ways in which this may arise, but the most common way apparently is for an egg to retain both of its X elements. Such an egg fertilized by a Y-bearing sperm produces an XXY embryo. Such an embryo retains throughout the entire development (cell-divisions) its two X's and its Y. There is evidence for this, obtained by Bridges, both from observation of the cells themselves and from the genetic behavior of such an individual.

In certain crosses between moths with different numbers and sizes of chromosomes, Federley, and Harrison, and Doncaster have shown that the cell of the hybrid contains half the number of each species, even with their characteristic size differences (Fig. 23). In crosses between different species of fish, where the size differences are quite conspicuous, it has been shown by Moenkhaus, Morris and Pinney (Fig. 24) that the embryonic cells may continue through their divisions to retain the characteristic chromosomes of both species. These hybrid cases are particularly significant; for the chromosomes derived from the father are in the foreign medium of the protoplasm of the other species. Nevertheless, in some cases they retain their own peculiarities, through successive cell generations.

Evidence That Homologous Chromosomes Mate with Each Other

That the mating of the chromosomes in pairs is not a haphazard process, but that each paternal chromosome

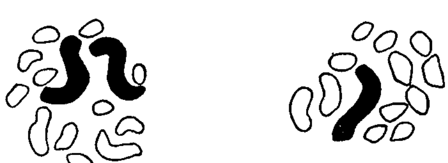

Fig. 25.—Female and male chromosome groups of *Protenor*. (After Wilson.)

mates with a definite maternal chromosome, has been established by evidence from several sources. In many species the chromosomes are of different sizes, and sometimes certain ones are markedly different in size from the others. In the bug *Protenor* the two sex-chromosomes of the female are conspicuously larger than the others (Fig. 25, *a*). When reduction takes place the sizes of

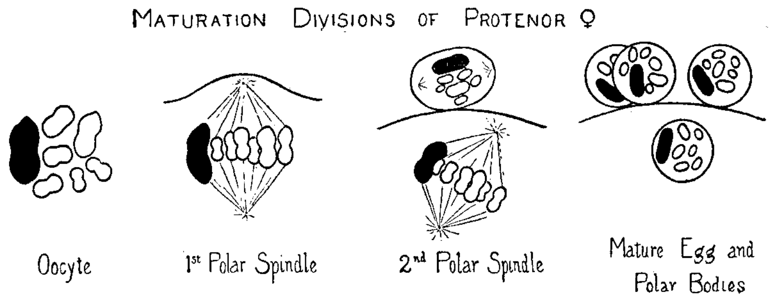

FIG. 26.—Reduced chromosome group; and extrusion of polar bodies in *Protenor.*

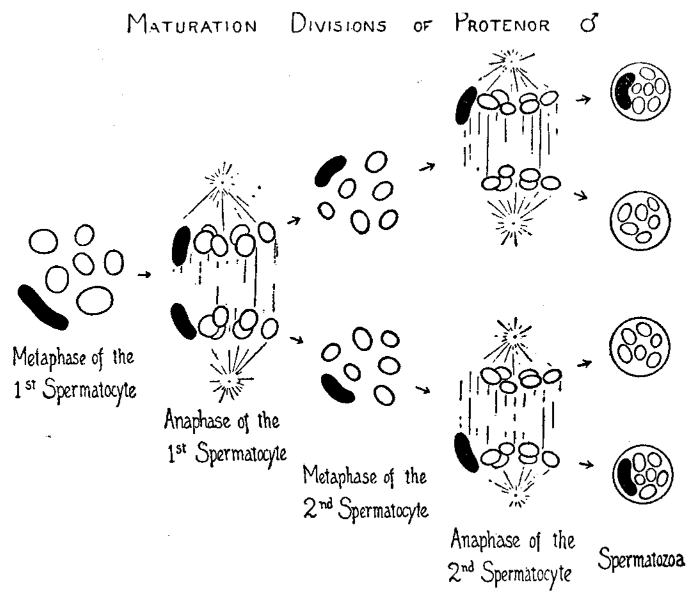

FIG. 27.—Reduced chromosome group of male; and spermatogenesis in *Protenor.*

the fused pairs show that these two large chromosomes must always unite with each other (Fig. 26). In the male of certain species, as in *Protenor* (Fig. 27), the sex chromosome has no mate, and therefore nothing to fuse with. Its size, after the others have conjugated (Fig. 27) shows that it remains single; while its failure to divide twice, as do the other chromosomes, corroborates the view that having no mate of its own it never combines with any other. At the other extreme, the two very minute chromosomes in several of the *Drosophila* species must have united to form the smallest chromosome of the reduced series (Fig. 28, *a–a′*, *b–b′*). In a few cases the X and the Y are different in size. When they fuse (in the male) the size of the fused mass is what

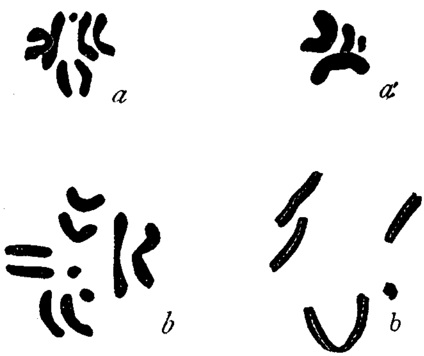

FIG. 28.—Diploid and haploid chromosome groups of *Drosophila busckii*, *a*, *a′*, and *D. melanica*, (*neglecta*) *b*, *b′*. (After Metz.)

is expected, viz., the sum of the masses of X and Y, and their subsequent separation into parts corresponding in size to the fused bodies supports the view that conjugation amongst the chromosomes is a very definite process. In the very exceptional case of a bug, *Metapodius*, there is a pair of small chromosomes called m's. When the other pairs enter the spindle the two m's come together, touch, and then separate, to pass to opposite poles.

RÉSUME

The evidence from studies of the maturation of eggs and sperm shows that the paternal and maternal chromosomes come together at this time in pairs, and subsequently separate, so that each egg comes to contain one or the other member of a pair. The same process takes place in the formation of the sperm-cells. It is obvious that if one member of any pair contains material that produces an effect on some character as one of the end results of its activity, and the other member of the pair contains a different material, the behavior of the chromosomes at the time of maturation supplies exactly the mechanism that Mendel's law of segregation calls for.