

CHAPTER III

REPRODUCTION AND THE LIFE CYCLE. INTRODUCTORY

“In both the rejuvenated infusorian and the fertilized egg-cell we see the onset of an energetic multiplication by cell-division which leads in the one case to the formation of a multicellular organism and in the other to a series of cell-generations. Morphologically, therefore, we must compare the sum of all the individuals of these generations to the multicellular higher organism that arises from the fertilized egg and itself in turn gives rise to eggs.”

BÜTSCHLI.¹

Growth and cell-division constitute the central phenomena in every process of reproduction; upon them, therefore, depend the genetic continuity of living organisms and the phenomena of heredity. The cyclical character so conspicuously shown in the life-histories of plants and animals generally prominently raises the question whether growth and cell-division are fundamentally rhythmic. We shall here briefly examine this subject from a general and elementary point of view in order to prepare the way for the cytological problems of reproduction to be considered in the four succeeding chapters.

It is obvious that the conditions on which the life-cycle depends are in part internal, in part external to the organism, but the relation between these has not yet become entirely clear.² We must also distinguish between the life-cycle of the individual and that of the species as shown in the succession of generations. In higher animals generally the individual life runs its allotted course in a certain sense automatically, in the absence of any corresponding cycle of external conditions. Given the normal conditions, and barring accident, every individual displays a succession of youth, maturity, old age and death as progressive phases of a process that goes forward without pause from the moment that the egg begins its development. In youth the constructive activities are in the ascendent, in age the destructive, while maturity is a period of relative physiological balance. To a certain extent the course of this process can be diverted by experimental modifications of external conditions; but sooner or later the end is inevitable.

In lower organisms many cases are known in which the life-cycle of the *species*, as shown by the alternation or succession of generations, is dependent upon changes of the external environment (for instance, in the

¹ *Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zelltheilung und die Conjugation der Infusorien: Abh. d. Senckenberg. Naturforsch. Ges. X, 3, 4, Frankfurt, 1876, p. 420.*

² For an interesting discussion of these relations see Jost, '07, '13.

rotifers, daphnids, or aphids, and in some of the Protista),¹ it is possible that such changes may play a leading perhaps even an exclusive part in the determination of the life-cycle.

In the highest animals (*e. g.*, in vertebrates) the life-cycle is a strictly sexual one which, so far as the race is concerned, may be said to begin and end with the egg (or sperm).² In many lower animals, and very generally in plants, the life-history is complicated by the occurrence of various forms of asexual reproduction, so that we may distinguish asexual cycles as well as sexual, often in regular alternation. At the opposite extreme, among the simplest forms of life, we find some cases such as the Bacteria and Cyanophyceæ in which no sexual cycle is yet certainly known. Some observers, it is true, have accepted the occurrence of a sexual process in certain of the bacteria in the form of a reunion of two incompletely separated daughter-cells; and an "autogamous" conjugation between sister-cells has been described in the yeasts and various other simple plants and animals examples of which are offered by the rhizopods *Actinophrys* or *Actinosphaerium* (p. 581). In case of the bacteria, however, it seems probable that the phenomenon that has thus been interpreted is probably not to be regarded as a degenerate process of conjugation, but an abortive cell-division.³

We are not yet, therefore, in a position to maintain that sexuality is a fundamental property of all living matter; nevertheless the occurrence of a sexual process in some form is all but universal among living things; and among Protista a definite sexual cycle may often clearly be distinguished, sometimes in alternation with an asexual cycle or cycles, as is seen in many sporozoa and in certain rhizopods (*e. g.*, *Polystomella*, Fig. 284). In free-living Protista such alternation may be correlated with external conditions of the environment, such as seasonal changes; in parasitic forms (*e. g.*, in the Hæmosporidia) with a change of hosts. The relation of the Protistan sexual cycle to that of multicellular forms is considered beyond (p. 238).

The group of problems that center in the sexual reproduction of higher organisms may be approached by a brief review of

I. THE REPRODUCTIVE PROCESSES IN GENERAL

Morphologically the reproductive processes of multicellular organisms may conveniently be distinguished as *somatogenic* and *cytogenic*,⁴ the former in-

¹ A valuable discussion of this question is given by Morgan ('07).

² "The egg is the mid-passage or transition stage between parents and offspring, between those who are, or were, and those who are about to be." Harvey, *De Generatione*, 1651, Trans., p. 271.

³ Dobell, '09, where further references to the literature are given.

⁴ These terms are due to Hartman ('04) and Waldeyer ('06).

cluding asexual multiplication by fission or budding in which the body itself divides to produce offspring that are essentially multicellular fragments of itself. Cytogenic reproduction (cytogeny) on the other hand, is effected by means of unicellular *germ-cells* which by growth and division may build up a new multicellular body. In the Protista this distinction does not properly exist, since the whole body is itself unicellular; here also, nevertheless, it is convenient to speak of reproduction as cytogenic.

Somatogenic reproduction is always asexual (*monogony*) while cytogenic may be either asexual or sexual (*amphigony*),¹ the latter characterized by a preliminary process of *syngamy* (*fertilization* or its equivalent, *conjugation*) in which two germ-cells or gametes unite and most commonly fuse to form a zygote. Asexual germ-cells or *agametes* are in general incapable of fertilization or activation by the sperm, and develop without a preliminary union of germ-cells, as is typically illustrated by the spores of higher plants.² Sexual germ-cells, on the other hand, have in general the power of union in syngamy, the product of their union being the *zygote*, with which a new life-cycle begins. In many higher organisms the gametes are incapable of development unless activated by process of syngamy. In other cases the gametes of the female (ova) may be activated without syngamy, and develop by the asexual process of *parthenogenesis*, which is analogous, broadly speaking, to the development of a spore. This phenomenon occurs naturally in many plants and animals, including especially the rotifers, lower crustaceans (daphnids, ostracodes), and insects (aphids, gall-flies, bees, etc.) and in some cases can be artificially induced by physico-chemical means in the sexual eggs of animals that do not naturally undergo parthenogenesis (p. 472). In many cases the ovum has completely lost the capacity for fertilization and has become a true agamete, developing only by parthenogenesis. Such ova occur in rotifers, daphnids, ostracodes, aphids and phylloxerans, and differ markedly from the sexual ova in mode of maturation and other characters. There is, however, reason to conclude that they have been derived from true sexual ova capable of fertilization (p. 794).

The relations between sexual reproduction and parthenogenesis as they occur in nature are widely varied. In some species parthenogenesis is a rare process which seems to be interpolated irregularly in the succession of sexual generations. This is often spoken of as *facultative* parthenogenesis. In others it becomes a constant feature of the life-cycle occurring only at certain periods, sometimes in regular alternation with the sexual process. In

¹ These terms are Haeckel's.

² The term spore is of broad and vague meaning, and some kinds of "spores" (e. g., *auxospores* of diatoms) are zygotes that result from a process of conjugation. Spores in the strict sense of the word do not occur in higher animals though found in many Protista.

this case the sexual generations are often morphologically different to such a degree as to have received different generic names (gall-flies); and thus arises a true alternation of generations. By further encroachment upon the sexual process parthenogenesis may come to constitute the major part of the life-cycle, the sexual forms only appearing at intervals following a certain number of asexual generations, as is the rule among rotifers, aphids, phylloxerans, daphnids and ostracodes.

In nearly all such cases the parthenogenetic eggs are incapable of fertilization and differ characteristically from the sexual eggs in mode of maturation (p. 467). This type of parthenogenesis is often called *obligatory*; but experiment has proved that the sexual process may in some cases be induced by changes in the environment¹ and has also shown that under suitable conditions the succession of parthenogenetic cycles may be greatly prolonged, perhaps indefinitely. The classical example of this is offered by the rose aphid, the normal life-history of which includes a series of parthenogenetic cycles during the summer months later terminated by a sexual process. If, however, the insects are cultivated in the greenhouse, the sexual cycle may be wholly suppressed, reproduction now continuing solely by parthenogenesis even through a series of years.² The process of senescence, it is true, still continues in each generation; for the life of the individual is limited as before; but the race is continued by a process in which syngamy plays no immediate part.

In rotifers, the researches of Maupas ('90, '91) showed that the appearance of the sexual forms may readily be induced by changes of temperature (later shown to produce this result by affecting the microorganisms on which the rotifers feed). The work thus initiated has more recently been developed with marked success by Whitney and by Shull³ with results slightly different in detail but similar in principle. When *Hydatina senta* is consecutively cultivated in old culture-fluid (infusion of horse-manure in spring-water) parthenogenesis may long go forwards without the appearance of sexual forms, but the latter quickly appear if the animals be transferred to fresh culture-fluid. Since this effect is produced even after filtration of the culture-fluid (the rotifers normally feed on flagellates) it is ascribed by Shull to dissolved substances that progressively accumulate in long-continued cultures. The same result follows from a suitable change of food. In *Hydatina* fed upon a uniform diet of the colorless flagellate *Polytoma* Whitney obtained 289 successive parthenogenetic generations, extending through nearly two years without the appearance of sexual

¹ This must not be confused with the determination of sex itself. Cf. p. 815.

² Kyber, '15, see Morgan, '07.

³ Whitney, '07, '10, '14, '16; Shull, '10, '10a, '11.

forms; but the sexual process could at any time be induced within a few hours by changing the diet from the colorless *Polytoma* to the chlorophyll-bearing *Chlamydomonas*. Results similar in principle were obtained in other rotifers (*Brachionus*, *Diaschiza*, *Diglena*, *Pedalion*).¹

In the foregoing cases the sexual process still forms a regular part of the life-cycle under the natural conditions of life, though parthenogenesis seems to play the main rôle. A step beyond this are cases in which parthenogenesis has assumed still greater importance, males being extremely rare or even entirely unknown, *e. g.*, among the free-living nematodes (Maupas) and in various insects, including certain species of walking-sticks (*Phasmidæ*), saw-flies (*Tenthredinidæ*), gall-flies (*Cynips*, *Rhodites*), scale-insects (*Coccidæ*) and Lepidoptera (*Psychidæ*). Similar differences in respect to sexuality and parthenogenesis between related species occur in various genera of plants. In *Alchemilla* (*Eualchemilla*) only a few species are sexual while many are exclusively parthenogenetic² and a somewhat similar condition seems to exist in *Rosa*.³ Both sexual and parthenogenetic species occur also in *Wikstræmia*, *Antennaria*, *Hieracium* and other genera.⁴

The evidence makes it highly probable that in all these cases the original mode of reproduction was sexual and that it has in greater or less degree been supplanted by parthenogenesis. This change seems to have taken place very readily, for even within the limits of a single genus some species may reproduce only by the sexual process, others by parthenogenesis only, still others by both. Remarkable examples of the plasticity of the reproductive phenomena in respect to these relations are offered among the free-living nematodes⁵ in which even closely related species, *e. g.*, of *Rhabditis* or *Diplogaster*, may show very striking differences. Many of the species are ordinary dioecious and strictly sexual forms, with the sexes in approximately equal numbers. Other forms are hermaphrodites having the morphological aspect of females (and formerly often described as such), but also producing sperm and being self-fertilizing; but these same species may have in addition true males, and sometimes also true females (*e. g.*, in *Rhabditis*). In most of the hermaphroditic forms true females are absent and the males, when present, are extremely rare, often only a small fraction of 1%. Beyond all this, some species are parthenogenetic, wholly or in

¹ It is a remarkable fact, determined by Maupas and confirmed by both Whitney and Shull, that the result of changed diet is not manifested in the individuals first affected but in their daughters, the effect being to induce the parthenogenetic production of daughters which produce sexual eggs, capable of fertilization. If unfertilized, these eggs produce males, if fertilized females, so that the definitive somatic effect first appears in the grandchildren of the forms originally treated.

² Murbeck, '01, Strasburger, '04.

³ Täckholm, '20.

⁴ See Winkler, '08a, b, Strasburger, '09, Tischler, '15, Ernst, '18.

⁵ Sharp, '95, Maupas, '00, Potts, '10.

part; and some show an alternation of generations between hermaphroditic and dioecious phases (p. 809). The evidence indicates that in all these various cases the primitive condition was dioecious, with equality of the sexes; and that hermaphroditism, parthenogenesis, etc., are derivative forms of reproduction accompanied, at least in some cases, by underlying cytological changes.

Varied relations between sexual reproduction and parthenogenesis have also been observed in insects of various orders. In the gall-fly *Rhodites rosæ*, for example, males are very rare, while they have not thus far been found at all in *Cynips Kollari*, a species which seems to reproduce solely by parthenogenesis.¹

Here too, such differences sometimes appear between nearly related species, e. g., in moths of the genus *Solenobia* (Seiler, '23) in which occur both modes of reproduction. In *S. pineti* the sexual forms predominate in northern Germany, with the sexes in nearly equal numbers, while towards the south the males become less numerous and in the neighborhood of Munich are of extreme rarity, parthenogenesis being the predominant type. In *S. triquetrella* these conditions are reversed, the sexual forms being very rare while only parthenogenetic forms are found over a large area in Germany, Austria, and Switzerland, (p. 805). The evidence clearly indicates in this case that the sexual and the parthenogenetic forms are distinct races that differ genetically and are not determined by external conditions.

The same fact appears with still greater clearness in cases where different strains or races of the same species differ in mode of reproduction, and are found to differ correspondingly in the underlying cytological conditions. A classical case of this is offered by the phyllopod *Artemia salina*, a cosmopolitan species which in general morphological type appears to represent a single species, but differs in the number of chromosomes. As shown by Artom ('11, '12, '21) this species includes two distinct races, one of which is strictly parthenogenetic, the other strictly sexual. The former, from Capodistria (near Trieste) and various other localities, produces only parthenogenetic eggs which give off only one polocyte and develop by diploid parthenogenesis, with 84 chromosomes. The second race, from Cagliari (Sardinia) and other localities, produces only sexual eggs, which give off two polocytes, undergo complete reduction (to 21 chromosomes) and upon fertilization develop with 42 chromosomes. Here, too, it is evident that the two races differ genetically, though their only constant morphological differences, apart from the chromosome-number, consist in the larger size of the nuclei and cells of the parthenogenetic race ("bivalens") and also the larger size of the body.

¹ For an account of the cytology of these forms see p. 803. See Hogben ('20a).

A close parallel to this is offered in plants by the long known case of *Chara crinita*.¹ This widespread species likewise occurs in two forms, one strictly parthenogenetic and diploid, and the other sexual and haploid. The diploid race, like a parthenogenetic aphid, undergoes no process of reduction and develops with the diploid number of chromosomes, 24. The sexual haploid race produces gametes having 12 chromosomes, which by their union must produce zygotes with 24 chromosomes. The resulting plants nevertheless have but 12 chromosomes, *i. e.*, the haploid number; hence it is practically certain that reduction here is zygotic, *i. e.*, occurs during the first two divisions of the zygote, as in *Spirogyra* or *Zygnema* (p. 491). Intermediate forms between the two races do not occur, and it is clear that they are genotypically distinct.

In both the foregoing cases there is strong reason to conclude that the parthenogenetic race has been secondarily derived from the sexual; and the same conclusion probably applies to many other cases of strictly or mainly parthenogenetic forms.

The relation of parthenogenesis to sex in these various cases will be considered later (p. 789). We here only refer to the remarkable variations which they sometimes show. In the homopterous insect *Trialeurodes vaporariorum*, for example, as shown by Williams ('17) and Schrader ('19), unfertilized eggs of the English form produce females, of the American form only males. A parallel to this is offered by the hymenopteran parasite *Trichogramma pretiosa*, the unfertilized eggs of which are said to produce males alone in the U. S. and in Europe either both sexes or females only (Howard and Fiske, '11). The explanation of these cases is still imperfectly known, but is no doubt to be looked for in underlying cytological conditions in the germ-cells.

Taken as a whole, the phenomena clearly prove that sexual reproduction may readily become much restricted or wholly dispensed with, even in relatively high organisms, and hence cannot be regarded as a fundamental necessity of continued life. A singular fact, which bears on this question, is that in the Hymenoptera generally the males appear always to arise from parthenogenetic eggs, the females from fertilized ones (p. 795).

II. SEXUAL REPRODUCTION. SYNGAMY AND ITS EFFECTS

The essential nature of the sexual process and the part which it plays in the life-cycle constitute one of the oldest riddles of biology, yet its final solution still eludes us. As far as we can see there is no *a priori* reason why, barring accident, one cell-division should not succeed another in endless succession. Such may actually be the case in some of the lowest forms of

¹ See especially Ernst, '18.

life, such as the Bacteria and Cyanophyceæ; but in vastly the greater number of living forms the succession of cell-divisions is periodically interrupted by a sexual process of which the essential act is syngamy or fusion of two cells (gametes) into one. This process is not in itself an act of reproduction (p. 580). It can at most be regarded as only an antecedent condition of reproduction, obligatory in the highest forms of life but in lower organisms often held in abeyance for long periods of time or even in some cases, it would seem, dispensed with altogether.

Why the sexual process should be necessary in any case is unknown; but we are able to perceive some of its morphological and physiological effects. Morphologically the most important effect of syngamy (together with the accompanying processes of maturation) is to bring about a periodic reorganization of the nucleus; but in parthenogenesis or endomixis (p. 244) an essentially similar reorganization is accomplished by other means. Physiologically we may recognize four distinct effects of syngamy. In higher animals the most conspicuous of these is the *activation or initiation of development* in the egg, thus inaugurating a new cycle of activity. It has long been held that an analogous effect is produced by the conjugation of unicellular organisms, and this view is strongly supported in certain cases by some of the most recent researches (Calkins, '19); but the fact has repeatedly been disputed. A second obvious physiological effect of syngamy is temporarily to bring into close association two previously separate lines of heredity; *i. e.*, *biparental heredity* by means of *amphimixis* (Weismann).

A third effect, less obvious than the foregoing, is an *increase of external diversity* in the offspring, conspicuously seen in the extremely variable offspring of the seed-progeny of many cultivated races of plants as compared with their relatively uniform offspring produced by asexual cuttings, grafts, tubers or bulbs. This is undoubtedly a recombination-effect resulting from the heterozygous nature of these races. A fourth effect, also most obvious in heterozygous forms of hybrids between different strains or races, is an *increase of vigor* in the offspring. This effect, superficially similar to the first of those enumerated above, is of interest in its bearing on the general theory of senescence and rejuvenescence. We cannot here consider *in extenso* the intricate physiological problems involved in these various effects of syngamy; but a brief survey of them is desirable as an introduction to the cytological phenomena to be considered in the following chapters.

1. Senescence, Syngamy, and Rejuvenescence

By an important group of investigators syngamy has been regarded as a direct cause of rejuvenescence, operating as the corrective to a progres-

sive process of senescence which otherwise would sooner or later culminate in natural death. Certainly when we consider only the higher plants and animals we must admit that the assumption contains at least a measure of truth; for in most cases the germ-cells die if they are not fertilized. Here, as Loeb has said, fertilization is a "life-saving act" which initiates a new cycle of assimilation, growth, and cell-division. It does not, however, follow that senescence and natural death are fundamental phenomena inherent in all living protoplasm, still less that syngamy is their only remedy. In some animals parthenogenesis may replace fertilization for long periods of time; in others it is the only known method of reproduction; in still others parthenogenesis may experimentally be substituted for fertilization, at least for a time, as in case of the rose-aphid (p. 229).

Further doubts are raised by experiments on normal somatic cells cultivated outside the body *in vitro*¹ and on tumor-cells transplanted from individual to individual. Fibroblasts obtained from the heart of a chick-embryo in 1912 were thus cultivated *in vitro* by Carrel and Ebeling for more than ten years and were still growing (1922) with undiminished vitality and without change of type. More than 30,000 cultures have thus been obtained from an original small fragment, the cells having passed through about 1900 generations; and had it been possible to preserve all the cells thus produced their combined volume to-day would be far larger than the sun.² Even more remarkable examples of this are offered by tumors (sarcoma) of rats and mice. One of these, discovered by Jensen in 1903, has been cultivated for many years in unbroken descent by inoculation from one animal to another, and has thus been spread to a large number of experimental laboratories throughout the world. This tumor is still growing with undiminished vigor though many generations of mice have meanwhile come and gone.³ The descendants of such a tumor—a sarcoma or connective-tissue tumor of the rat, discovered in 1908, also by Jensen,—are still actively growing in the Crocker Research Laboratory of Columbia University after more than twelve years of continuous transplantation from rat to rat.⁴ Accurate studies on this tumor during the past seven years have shown that, if we assume an unlimited supply of rats and the ability to make the transfer of all of every tumor at intervals of 23.7 days

¹ That embryonic cells isolated from their fellows may continue to multiply and differentiate was shown by the early work of Chabry, Driesch, Wilson and others (p. 1048). The continued cultures of such cells *in vitro* was successfully accomplished by Leo Loeb ('97, '07, etc.) and especially by Harrison ('07, '10, etc.) and later much extended by the work of Carrel and Burrows ('11), M. and W. Lewis ('11), Ebeling ('19) and others.

² Ebeling, '22.

³ See Leo Loeb ('01, '02, '08, '15, '17, etc.).

⁴ I am indebted for the calculations based on these data to the statistician of the laboratory, Miss Mary R. Curtis.

the entire mass of tumor produced in 21 months would be 1.7 times the space occupied by the solar system as far as the orbit of Neptune. The mass at the end of three years can only be stated in units represented by the distance of the sun to the polestar. Such a statement impresses us with the limitless power of increase of living matter by growth and division, and with the fact that even in the cells of mammals this power seems under proper conditions to undergo no perceptible decrease in the complete absence of any process of syngamy.

The same fact is brought home to us by the history of certain cultivated plants which have systematically been propagated for very long periods of time by tubers, cuttings, grafts,¹ or other strictly somatogenic processes without impairment of vigor. A climax is reached in those species which have become sexually completely sterile and reproduce exclusively by parthenogenesis, apogamy or other asexual processes, a condition rare among animals but not uncommon in higher plants (p. 230). Such facts support Weismann's contention that senescence of the somatic cells, though real, does not result from an inherent property of living protoplasm as such, but is due to secondary conditions. The one-celled organisms, in Weismann's view,² undergo no process of senescence and death; these organisms he believed to be endowed potentially with unending life; but here apparently his conclusion was pushed too far, as we shall presently see (p. 246).

The causes of senescence in higher organisms have long been a subject of inquiry. Herbert Spencer early suggested that it arises from a progressive increase of protoplasmic stability, *i. e.*, "an approach towards molecular equilibrium,"³ in which condition energy is less readily liberated by chemical action. Syngamy operates to overcome this condition by the mixture of two slightly different protoplasts, thus rendering the protoplasm more labile, "reëstablishing active molecular change in the germ" by which energy is set free, and also leading to increased variability. It may be doubted whether any later writer has offered a more satisfactory interpretation of senescence, though many have endeavored to render it more specific. Weismann⁴ ascribed senescence to progressive *differentiation* by which the cell gradually loses its plasticity, and reproductive power; and this view has been widely adopted by later writers.⁵ Minot, and later R. Hertwig⁶ sought for a quantitative explanation of the phenomena, as-

¹ In some cases for centuries; *e. g.*, in the willow, poplar, sugar-cane or the cultivated banana (see Jost, '07).

² Weismann, '81, '83, '84, '13.

³ *Principles of Biology*, 1866, Am. Ed., p. 234. It is interesting to recall that in connection with this Spencer emphasized the colloidal nature of protoplasm.

⁴ *Life and Death*, 1883.

⁵ See Kassowitz ('99), Enriques ('07, '09), Child ('11, '14, '15), Conklin ('12, '13), Schleip ('15), etc.

⁶ See Minot ('90, '95 '08), R. Hertwig ('89, '99, '02, '03, '05, '08).

cribing senescence to a disturbance in the normal karyoplasmic ratio, *i. e.*, in the equilibrium between nuclear and cytoplasmic mass. Minot assumed this to result from a progressive increase of the cytoplasm, accompanied by differentiation or *cytomorphosis* with the products of which the cytosome gradually becomes overloaded.¹ This condition, in his view, is counteracted by the cleavage of the ovum, in which he believed the nuclear material to be rapidly increased while the cytoplasm remains nearly stationary.

Hertwig's assumption was in one principal respect the opposite of Minot's, senescence being ascribed to a progressive increase in the nuclear volume as compared to the cytoplasmic, a result counteracted either by an extrusion of chromidia from the nucleus (in Protozoa) or by the nuclear reorganization that takes place in the course of maturation and syngamy. In the case of multicellular forms Hertwig regarded both sperm and egg as senescent or in a state of depression, owing to their relatively abnormally large nuclei and an accompanying accumulation of differentiated components in the cytosome. This condition is overcome, in part by the liberation of a large amount of nuclear substance during maturation (a well-known fact)² in part by syngamy and cleavage. The first of these three processes reduces the size of the egg-nucleus (p. 259) far below the normal, while the second and third gradually restore it to the normal. Hertwig, like Minot, thus assumed that cleavage involves a large increase of nuclear material.³

It cannot be said that either of the above two hypotheses has had much result, except in so far as both recognize the importance of differentiation as a cause of senescence. So far as the karyoplasmic relation is concerned, facts may be cited in favor of each. The great increase in size of the cytosome as compared with the nucleus in many tissue-cells of both plants and animals has long been recognized. Eycleshymer, for instance ('04), found that in the striated muscle-cells of *Necturus* the increase of plasma-volume was about ten times that of the nuclear in the growth of larvæ from 8 mm. long to the adult condition, and an even greater increase has often been noted in the growth of plant-cells. On the other hand, the relatively large nuclei of both egg and sperm in higher forms and in the stages of depression in various Protozoa (*e. g.*, in *Actinosphaerium*) are also observed facts. Later studies have, however, shown that some of the assumptions of Minot and Hertwig cannot be sustained.

It has been shown that the karyoplasmic ratio is in some cases subject to wide variations even at the same period of the life-history (p. 731), and

¹ '08, pp. 161 ff.

² *Cj.* pp. 328, 356.

³ For an interesting development of Hertwig's views see Popoff ('08).

that in these cases the adult tissue-cells show no marked increase of cytoplasm over nucleus as compared with the blastomeres.¹ Concerning the increase of nuclear volume during cleavage assumed by Minot, Hertwig and other writers the existing data are contradictory. Godlewsky ('08) found in sea-urchins that the nuclear volume almost doubles at each cleavage up to the 64-cell stage, but thereafter the increase is very slight up to the 256-cell stage. Godlewsky assumes, however, that the nuclei grow richer in "chromatin" during this period. Erdmann ('08) found in *Paracentrotus* (*Strongylocentrotus*) that in the pluteus stage the ratio of chromatin to plasma is about seven times greater than at the beginning of development. On the other hand, Conklin found in *Crepidula* that the average nuclear growth in *Crepidula* up to the 32-cell stage is not more than 5 to 9% for each division and is greatly decreased subsequently. This is perhaps in part explained by the fact, determined by Erdmann ('08) and by Baltzer ('08), that both the nuclei and the chromosomes grow progressively smaller as cleavage advances. In sea-urchins Erdmann found that the chromosomes in the pluteus had only about $\frac{1}{4}$ the volume of those of the first division; and Conklin observed a similar decrease of size in the chromosomes of *Crepidula*.²

These various facts show on how precarious a basis rest theories of senescence and rejuvenescence which refer these processes to changes in the karyoplasmic ratio. On the other hand, nearly all modern observers have emphasized the importance of those underlying processes of metabolism that are expressed in the phenomena of differentiation and the resulting accumulation in the cytosome of relatively inactive products. Child refers senescence to a decrease in the rate of metabolism (which may result from any cause) while conversely rejuvenescence results from an increase in the rate, due to "the removal in one way or another of the structural obstacles to metabolism" ('11, '15). This conclusion, in substance closely akin to that of Spencer, Weismann and other early writers, is probably well founded; but unfortunately none of these conclusions seems to touch the bottom of the problem. The long-continued cultivation of tissue-cells *in vitro*, or of tumor-cells by successive transplantations, shows that even differentiated cells may live far beyond the normal span of life under suitable conditions. There is much force, therefore, in the contention of Pearl ('22) that the senescence of higher organisms is a consequence of the complex balance between the mutually dependent cells of which they are built up, a balance readily upset by pathological changes that may take place in any particular part and which may be beyond repair by the normal regulatory powers of the organism.

¹ See Conklin, '12.

² Marcus ('06), Erdmann ('08), Baltzer ('08), Conklin ('12).

Physiological Effects of Conjugation in Protista. While "rejuvenescence" as a consequence of fertilization is an obvious fact in the higher plants and animals, such is far from the case in Protista; here, indeed, the physiological effect of conjugation constitutes one of the most ardently debated problems of general biology.¹ It was suggested already by Dujardin (1841) that simple cell-division in Protozoa cannot go forward indefinitely, and a similar notion was entertained by Balbiani ('60) and by Claparède and Lachmann ('60). H. Spencer raised the same question, but the problem first appeared in clearly defined form with the classical works of Bütschli ('76) and Engelmann ('76). These observers produced experimental evidence, drawn from the study of ciliates, to show that after a certain number of divisions there begins a gradual process of degeneration which ultimately leads to death unless counteracted by a remedial process of reorganization and rejuvenescence (*Verjüngung*) that is brought about by conjugation. Enlarging this conception, Bütschli pointed out the close analogy of the individual metazoön and the protozoan sexual cycle. In both cases, he urged, syngamy inaugurates a new cycle of growth and division. In the protozoön the resulting cells separate to lead independent lives, in the metazoön, they remain in close association to form a multicellular individual;² but in each case the cells of the cycle undergo progressive senescence, which culminates in death if not counteracted by conjugation or fertilization. Syngamy was thus regarded by Bütschli as a *conditio sine qua non* of continued life, a conclusion also supported by Engelmann ('76) and Balbiani ('82). It was challenged in Weismann's suggestive essay on life and death (1881), which offered a formidable argument against natural or inherent senescence in the Protista. Under suitable conditions, urged Weismann, the unicellular organisms are capable of indefinitely continued growth and division. Senescence and natural death in multicellular organisms were regarded by Weismann as secondary phenomena resulting from a diminution or loss of growth and reproductive power on the part of the somatic cells; and this loss he ascribed to their specialization and differentiation for other functions.

The celebrated researches of Maupas ('88, '89) on the ciliate Infusoria seemed at first to bring conclusive experimental confirmation of Bütschli's and Engelmann's conclusions; and similar results were reached by R. Hertwig and by many other observers;³ but later studies revealed many complicating factors which raised fresh doubts concerning their validity. Mau-

¹ A critical review of this subject down to 1914, is given by Dobell ('14). See also Calkins ('19, '20), Jennings ('21), Pearl ('22).

² Cf. pp. 103, 1031.

³ R. Hertwig, '89, '00, '04, etc.; Joukowsky, '98; Simpson, '01; Calkins, '02, '04, '06, '07, '13; Calkins and Cull, '07; Calkins and Gregory, '13; Gregory, '09; Woodruff, '05; Moody, '12, etc.

pas found that in pure cultures of various ciliates (*Stylonychia*, *Oxytricha*, *Leucophrys*, and others) the animals passed through a definite cycle of changes comprising, first, an "agamic period" of adolescence and vigor during which conjugation does not take place; then one of "puberty" and sexual maturity or "eugamic" condition, during which conjugation readily occurs; finally, a period of age or "senescence," when the animals become sickly, degenerate and finally die of old age. The effects of senescence

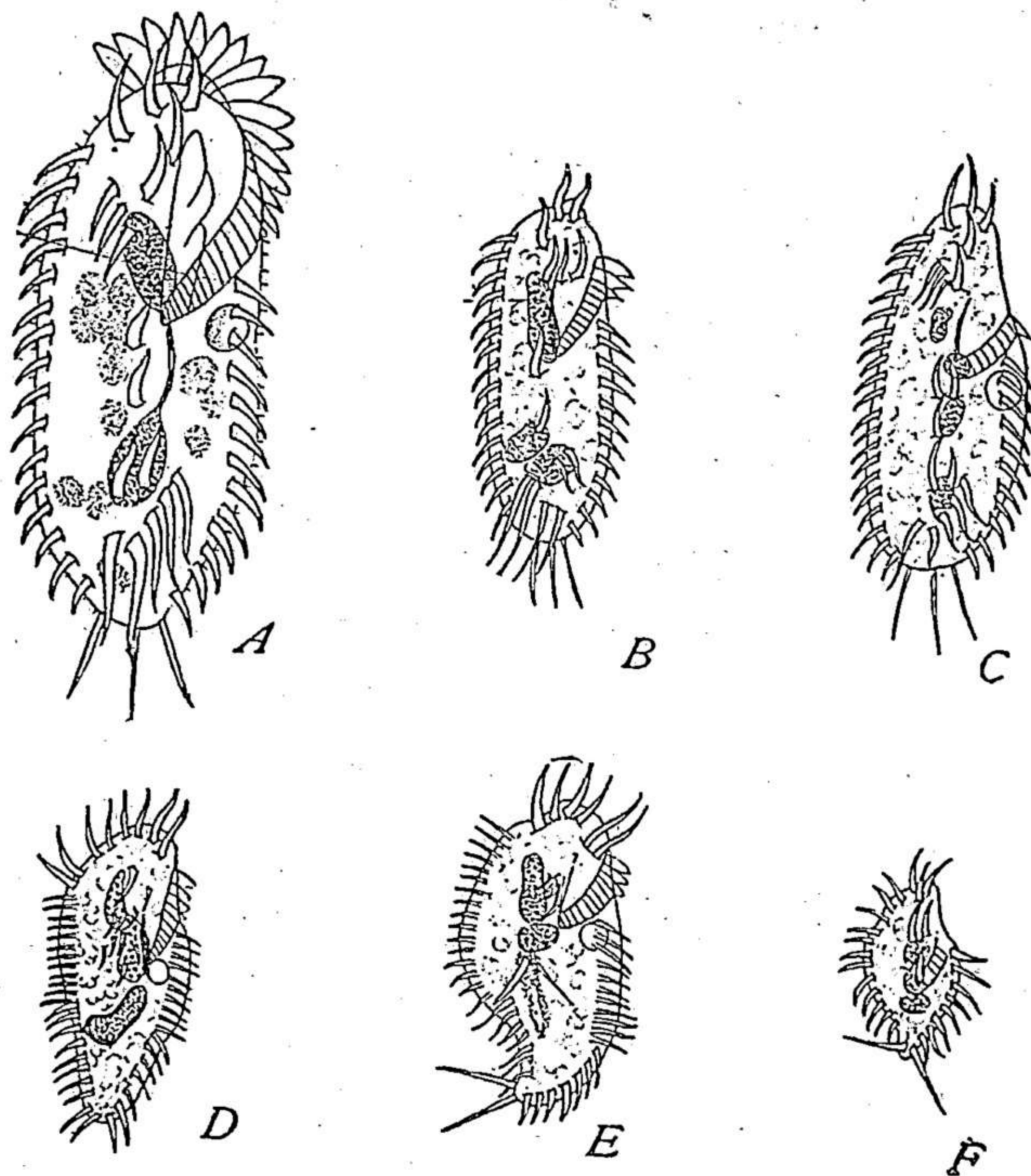


Fig. 97.—Progressive degeneration in *Stylonychia pustulata* (MAUPAS).

A, first stage, disappearance of micronuclei, slight reduction of size; B, C, second stage, fragmentation of macronucleus, disappearance of frontal membranellæ; these animals no longer feed; D, E, individuals in second stage shortly after division; F, last stage, shortly before death.

appear in structural changes in the protoplasm, reduction of size, and degeneration of the nuclear apparatus, commonly involving the disappearance of the micronucleus and the fragmentation of the macronucleus. This is followed by reduction or disappearance of the anterior appendages, such as the membranellæ and mouth-parts of the heterotrichous forms, by external deformities, inability to take food, rapid decrease in size and finally by death (Figs. 97, 98). Maupas believed that in its earlier stages senescence may be counteracted by conjugation but later is irremediable. The length of the cycle as measured by number of divisions was found to vary with the species; in *Stylonychia pustulata* the number was 316, in *S. mytilus* 319, in *Oxytricha* and *Onychodromus* 320–330, in *Leucophrys patula*, 660.

The length in time varied materially with conditions of food and temperature, as was to be expected. In a culture of *Stylonychia pustulata* the agamic youthful period extended to about the 130th division (when the first conjugations appeared), the eugamic or mature period to the 170th, the aged or senescent period to the 316th, when the race died out. The effect of conjugation was tested in another culture by isolating an individual in the mature state, and allowing it to conjugate with a wild individual from another stock. The offspring of the ex-conjugants continued to divide for

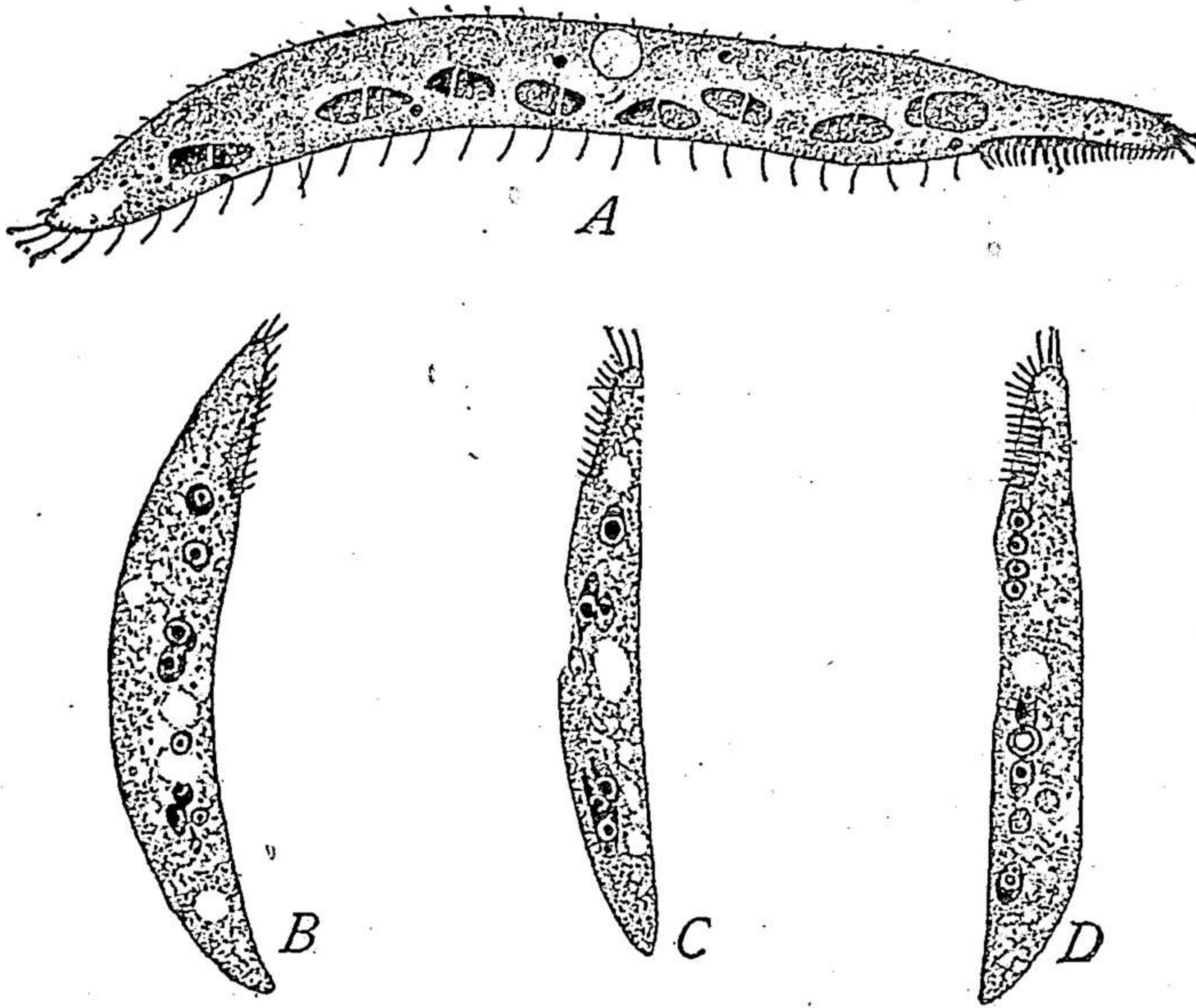


Fig. 98.—*Uroleptus mobilis* (CALKINS).

A, Normal individual, with eight macronuclei and four micronuclei; B, C, D, dwarfed and degenerate individuals (drawn to the same scale) in the last stages of senescence; B, from "F series," 315th generation; C, from the "C-series" 316th generation, a sister-cell of the last individual of the cycle, showing degeneration of macro- and micronuclei.

more than 300 generations before death supervened; while the control animals, prevented from conjugation, lived for only 89 generations.

Results similar in all essentials to the foregoing were obtained in *Paramecium* and various other Infusoria by R. Hertwig, Calkins, Doflein, Popoff, Joukowsky, Simpson and many others who found that under standardized conditions of food, temperature and other external conditions the life of these Protozoa runs in cycles, periods of activity being followed by periods of *depression* (Calkins) followed by death, if conjugation does not take place. The length of such cycles was found to vary widely in different species and even in the same species under different conditions. In Calkins' original cultures of *Paramecium* the periods of depression occurred at intervals of about six months, the number of divisions being approximately 200, or in some cases considerably lower (Fig. 99).

Maupas and his successors showed that the animals reach sexual maturity long before marked depression sets in; in the final period of the cycle, indeed, they are often incapable of conjugation. Even at the period of maturity conjugation does not necessarily take place. The animals have only attained a condition in which conjugation may readily be induced by a change of physiological conditions, most readily by a sudden decline in nutritive conditions that have previously been favorable—a fact that seems to be parallel to the incitement of sexual reproduction in plants by checking the food supply. In like manner the classical researches of Klebs¹ have shown that conjugation in various simple algæ, such as *Chlamydomonas*, *Spirogyra* or *Ædogonium* may be incited by a reduction in the quantity of in-

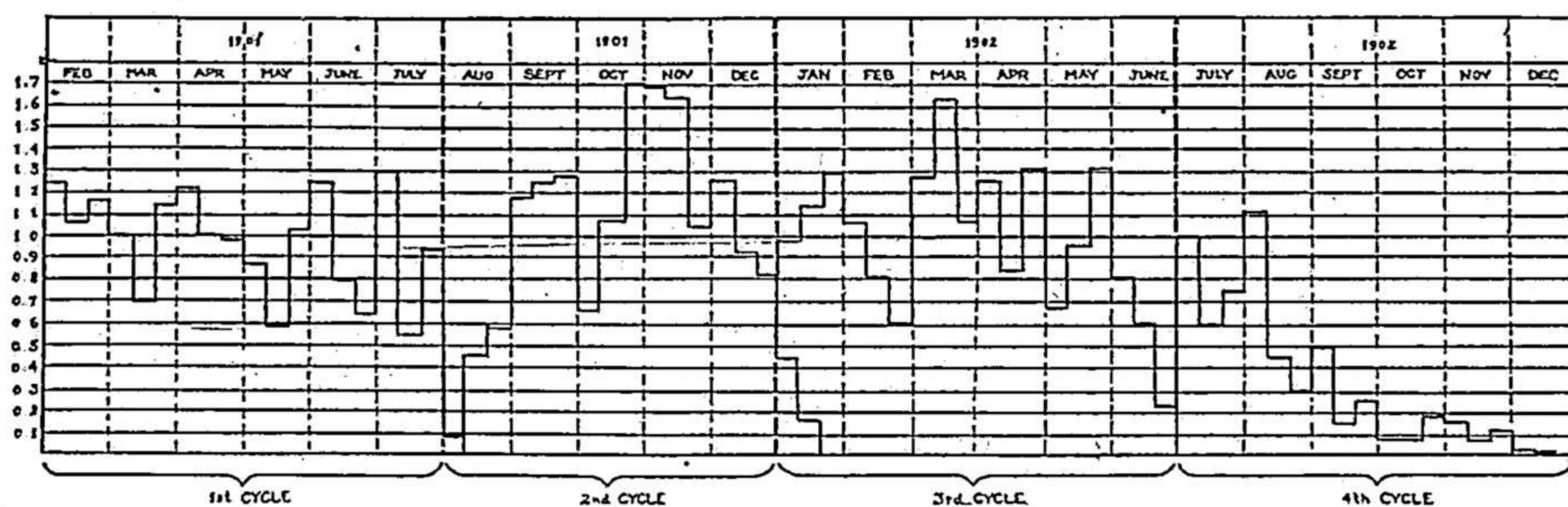


Fig. 99.—Curve of complete history of a culture of *Paramæcium caudatum*, extending over 23 months; steps in the curve are 10-day mean division-rate, ordinates daily division-rate; abscissas indicate time by 10-day periods; limits of cycles (when change of diet occurred) indicated by heavy dotted lines. The lines of the curve carried to the base indicate dying out of the control culture when not stimulated by change of diet. Four cycles are shown, each of 5-6 months, and 200 divisions (CALKINS).

organic salts accompanied by the action of bright light, or in some cases by the addition of sugar solution.

Continued research soon revealed the existence of unsuspected factors. The duration and character of the life-cycle were found to be markedly affected by external factors, such as food, accumulated waste products, and the like (Calkins, Enriques and others). It was found that the larger life-cycle (as limited by conjugation) is complicated by the existence of minor asexual cycles or "rhythms," analogous to the parthenogenetic cycles of higher animals (rotifers, aphids, daphnids, etc.), in some cases apparently independent of external conditions (Woodruff, Erdmann, R. Hertwig) in others apparently determined by them (Woodruff, Jollos). It was found, finally, that in respect to the duration and character of the life-cycle wide differences may exist even between different races or strains of the same species (Jennings, Calkins); and here was found a partial explanation of the conflicting data of earlier observers.

¹ Klebs, '96, etc., reviewed in Klebs, '04, '13.

These researches can here be reviewed in only the briefest manner. Calkins ('02, '03-'04) discovered that in cultures of *Paramecium* cultivated in hay-infusion the periods of depression might for a time be overcome without conjugation by a change of culture-medium (e. g.: from hay-infusion to beef-extract),¹ or by temporary treatment with simple salt-solutions such as potassium phosphate or chloride. Control animals, not thus treated, died out at the end of about six months (200 generations); while those placed under the new conditions entered upon a new cycle. This was repeated for three succeeding cycles of six months, the depression in each case being overcome by similar treatment. The race was thus kept alive for 23 months, when in spite of every effort it died out at the 742d generation. Recognizing the close analogy between this effect and artificial parthenogenesis of the animal egg (pp. 472, 476) Calkins suggested that many different kinds of stimuli might be substituted for normal conjugation and produce like results—"It is often surprising to see what slight stimuli are required to bring these about; and the conclusion seems undeniable that such changes, and similar stimuli, may operate in the natural habitats of Infusoria. If they do, Weismann's conclusions regarding "immortality" of Infusoria may be justified."²

The subsequent long-continued studies of Woodruff ('05-'21), demonstrate that when *Paramecia* are cultivated in infusions of various organic materials taken at random from natural ponds they may be kept in a flourishing condition, without fatal periods of depression and *without conjugation* for very long periods. Woodruff's main culture of *Paramecium aurelia* was thus maintained without conjugation, for a period of more than thirteen years in the course of which more than 8,000 generations were passed. This result is not due, as first suspected, to an inability of this particular race to conjugate, for conjugation was induced in it after six years (4102d generation) and again six and a half years later. So far as this particular race is concerned, therefore, syngamy evidently is not an indispensable condition of continued life.³

Meanwhile it was demonstrated by several observers,⁴ that periods of depression like those described by the earlier observers, may be due to the prolonged action of bacterial toxins, excretion-products, or similar unsuitable conditions. The suspicion thus arose that the cyclical aspect of infusorial life in artificial cultures may be due to this cause rather than to an intrinsic tendency towards senescence and death.

Further complications arose from conflicting results concerning the

¹ These ciliates feed on the bacteria that develop in such infusions.

² '02, p. 138.

³ See also Enriques ('16) on *Glaucoma* and Hartman ('17, '21) on *Eudorina*.

⁴ Enriques, '03-'10; Popoff, '09; Woodruff, '11; Baitsell, '12, and others.

effect of conjugation on the division-rate, which by many investigators had been taken as an index of vitality. Maupas himself denied the original conclusion of Bütschli and Balbiani that conjugation increases the division-rate ('89, p. 504); R. Hertwig ('89) even found the rate in some cases diminished. Hertwig later pointed out that the relation between syngamy and the division-rate differs in different organisms; in some Protozoa it is increased by conjugation, in others unaffected or somewhat retarded, in still others conjugation is followed by complete cessation of division for a considerable period (flagellates, rhizopoda), and the same is true of many lower plants (*Spirogyra*, desmids, *Mucor*, etc.). Some later observers found distinct evidences of increased division-rate following conjugation, in particular Calkins; but this in turn was contradicted by Jennings ('13, '21) whose extended researches on *Paramecium* not only produced no evidence of an increased division-rate after conjugation but actually showed in many cases an increase of mortality among the ex-conjugants. This led him to a sweeping denial that conjugation restores the declining rate of division during periods of depression and to a decided scepticism concerning the whole theory of senescence and rejuvenation in the Protozoa. Jennings, indeed, considered the evidence overwhelming that the degeneration observed in laboratory cultures is "simply a result of bad conditions." His work led also to the interesting discovery that in the same species exist distinct races or strains differing characteristically in respect to the conditions of conjugation. Some of these races conjugate very rarely (as in Woodruff's culture), others frequently and with great readiness. This shows with how much caution we must regard all results based on material of which the genetic nature is not fully known.¹

With the publication of these results the reaction from the conclusions of Bütschli, Maupas and Hertwig reached its climax; but the pendulum of opinion now began to swing in the opposite direction. This movement began with the important discovery of *endomixis* or natural parthenogenesis in ciliates, a process correlated with the minor asexual cycles or reproductive rhythms referred to above. The time-duration of such rhythms differs considerably in different races but in each race is nearly constant even under considerable variation of external conditions, but the number of divisions seems to be more modifiable by such means (Woodruff, '17). In *Paramecium aurelia*, rhythmic periods of depression occur at intervals of about four weeks or forty generations (Fig. 100), while the major cycle of earlier observers extended through three to six months, or from 200 to 600 generations (Woodruff, '05-'07).

¹ Similar differences in respect to the tendency to conjugate were observed by Calkins and Gregory ('13) among the offspring of single ex-conjugants, reared under identical conditions.

Cytological study proved¹ that recovery from the periods of depression between successive rhythms is *accompanied by a process of reconstruction of the nuclear apparatus*, to which was given the name of endomixis in contradistinction to the *amphimixis* effected by syngamy. In both processes the old macronucleus breaks down, disappears and is replaced by one derived from the micronucleus; but in endomixis no process of karyogamy is concerned. In these respects, as will later be shown, amphimixis and endomixis show a close analogy, respectively, to fertilization and parthenogenesis in the multicellular organisms.² Woodruff ('17) has shown that after cessation

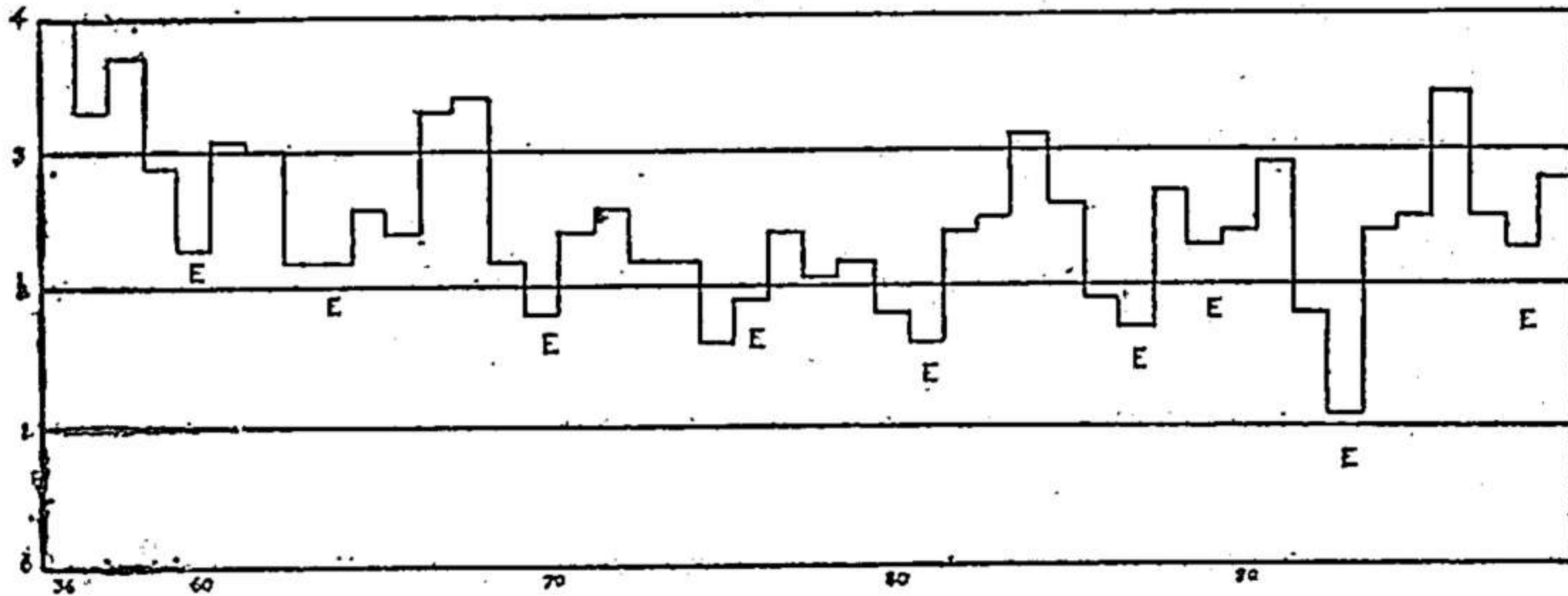


Fig. 100.—Curve showing endomictic periods in *Paramaecium aurelia* in varied culture-medium, laid out in 5-day periods. Ordinates show number of divisions daily, abscissas, number of 5-day periods. Time of endomixis indicated by E, E, etc. Total length of time 222 days. The endomictic periods are from 20 to 30 days in length, and average about fifty divisions each. (WOODRUFF.)

of the endomictic process (such as finally occurred in some of his cultures) the culture soon dies out. It is probable, therefore, that endomixis is necessary for the continued life of the race; and that endomixis and conjugation play essentially the same physiological rôle.³

This discovery placed the whole problem in a new light. *Paramaecium* and its allies, evidently, is an unfavorable form for experimental analysis, owing to the complication of the sexual cycle by periodic parthenogenesis (endomixis) and by its susceptibility to variations in the environment. A more favorable object is offered by the hypotrichous ciliate *Uroleptus mobilis*, a form in which, as in some other hypotrichs, endomixis takes place only during encystment, conjugation only during the free swimming condition. Here, therefore, the two processes are kept separate by nature. The results of an extended experimental study of this form by Calkins ('20) show conclusively that conjugation here operates as an activator of cell-division and a renewer of vitality. As is now customary in such experiments the many pure lines studied were of known pedigree, all alike being descendants of a *single individual*, isolated immediately after conjugation

¹ Woodruff and Erdmann, '14.

² The cytological phenomena of endomixis and conjugation are considered more in detail in Chapter VII (p. 613).

³ Woodruff and Erdmann, '14, pp. 490-492. See also Calkins, '02, and R. Hertwig, '00-'04, '14

and carried on in isolated cultures under identical conditions of food, temperature, and other conditions. All therefore consisted of the same protoplasm subjected to the same external conditions. Descendants of this individual were separated into many separate pure lines each starting with an ex-conjugant. In spite of complete uniformity in the external conditions, such lines were found to vary markedly in vitality as measured by the rate of growth and division. All alike, however, show a gradual slackening of the rate as time goes on; and if conjugation does not occur finally die out.

(1) In round numbers, the length of life in the stronger lines lay between 200 and 300 days, comprising from 250 to 350 divisions. Calkins distinguishes somewhat arbitrarily, between an earlier period of "youth," in which the mean division rate is more than ten for a period of ten days, and a period of "age," in which the rate is lower than ten. The period of youth and maturity (the mean of sixteen pure lines) was 139 days or 214 divisions; that of age 94 days or 65 divisions. Conjugation tests showed that in all the lines sexual maturity (*i. e.*, capacity for conjugation) is not ordinarily attained before the fiftieth day, appears in full vigor from about the sixtieth day, and persists until near the end of the cycle.

(2) In lines prevented from conjugation the division-rate begins to slacken in the second 60-day period, drops somewhat further in the third 60-day period, and very markedly in the fourth and fifth. The animals now become weakened, degenerate and dwarfed, the micronuclei disappear and the macronuclei show degenerative changes; but no evidence of cytoplasmic degeneration appears (Fig. 98). The power of division is finally lost and death invariably follows, although such degenerate individuals may live for a month.

(3) If at any time during the period of sexual maturity (*i. e.*, after the first 60 days) conjugation takes place (between members of the same series) the division-rate is nearly or quite restored to the original maximum and a new cycle is originated which runs the same course as before. This effect is the more striking the later the period at which conjugation is induced, as will be seen from the curves in Fig. 101 which shows a primary cycle (A) and two of its offshoots, H and J, taken at different periods. In every case the ex-conjugant soon returns approximately to the normal ex-conjugant rate (15-22 divisions per 10-day period), and the period of death is delayed, in H for about 120 days, in J for more than 100 additional days. By taking off successive lines in this manner, each initiated by an act of conjugation, the life of the culture, the product of a single original individual, has thus been extended from the normal limit of 200-300 days to more than five years.

If, therefore, endomixis is here really excluded by the conditions of the

experiment, there seems to be no escape from the conclusion (1) that in the absence of either conjugation or endomixis the protoplasm of this species, placed under standardized and constant external conditions, undergoes a progressive change, involving successively a period of immaturity, sexual maturity, decline, degeneration and finally death; and that (2) conjugation

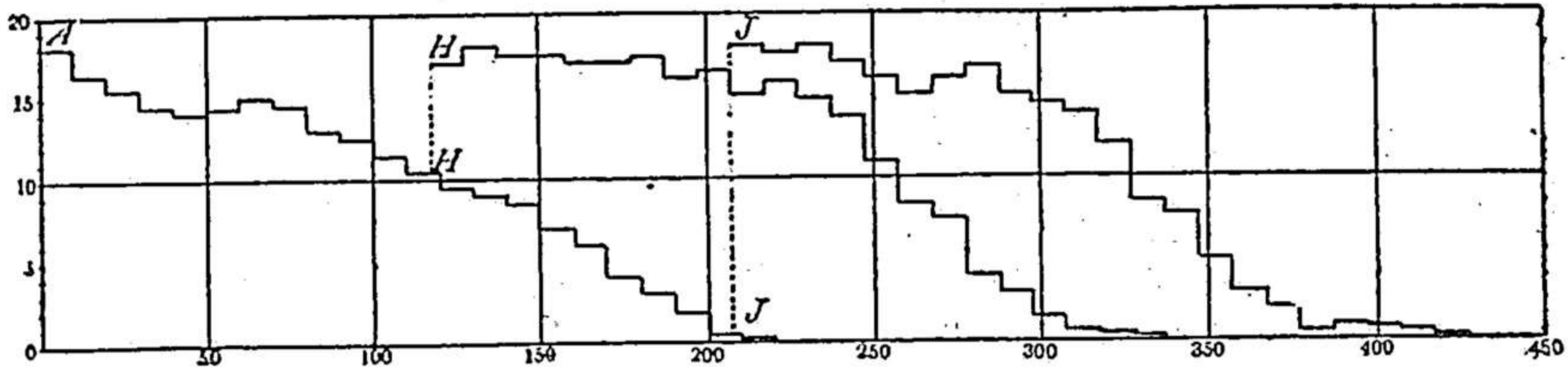


Fig. 101.—Curves showing “rejuvenescence” following conjugation in *Uroleptus* (adapted from CALKINS).

The ordinates show the mean division-rate per ten days, the abscissas the time in days. Each curve begins with an individual isolated immediately after conjugation; its meeting with the base-line shows the death of the line.

A, the original line (220 days); H, second line taken from the original one and permitted to conjugate after 116 days, with life prolonged to 335 days; J, third line taken from A near the end of its life (205 days), with extension of life to 428 days.

initiates a new cycle of activity by a restoration of the waning metabolic activities involved as expressed in growth and division.

There are still points to be cleared up in these results. Calkins's data show that under the conditions of these experiments there has been a slight falling off in the mean rate of division; in other words, conjugation has not in the long run been able to maintain the original rate unimpaired. Although these cultures have now been carried on for more than five years under the artificial conditions of the experiment it still remains to be seen whether they can be indefinitely prolonged. Further, until more adequate demonstration is produced that endomixis is excluded by the conditions of the experiment a certain reserve on this point seems justified. Nevertheless the outstanding fact seems to be inescapable that, in this particular case at least, *conjugation does in fact renew the failing metabolic activities of the cell*, and initiates a new cycle of growth and division. How this effect is produced is not certainly known; but since it seems also to result from endomixis or parthenogenesis there is considerable ground for the conclusion that it is connected with a reorganization of the nucleus that takes place during the processes of maturation. Such an explanation, however, would be inadequate in the case of higher organisms; for in most of them the maturation-process does not in nature lead to activation unless followed by syngamy.

We should be cautious in any attempt to extend these results to other ciliates or to Protista generally. In certain species of ciliates races or strains have been discovered that are devoid of micronuclei and hence

are unable to go through with the normal process of conjugation. Such a race was found by Dawson ('19) in the hypotrich *Oxytricha hymenostoma* and carried on for more than two years without the occurrence of true syngamy (though plastogamy frequently occurred). More convincing are the experiments of Hartmann ('21) on the phytoflagellate *Eudorina elegans*, a form in which the gametes are markedly heterogamous, and hence readily recognizable. This form was cultivated under carefully controlled conditions for 1300 generations, covering a period of five years, without the occurrence of syngamy or discoverable endomixis, and without undergoing periods of depression and regulation so long as the normal conditions were maintained. The climax appears in such organisms as the bacteria and the Cyanophyceæ in which no process of syngamy is yet known.

From all this emerges the large fact that the original theory of a universal, fundamental and innate tendency to senescence and death on the part of living protoplasm can no longer be maintained. The opposite conclusion would seem nearer the truth, namely, that barring accident or secondary limiting conditions, many forms of protoplasm are capable of indefinitely continuing life. In the vast majority of living organisms, however, such limiting conditions undoubtedly occur. In higher organisms generally it is only the germ-cells, as Weismann urged, that are endowed with an apparatus of periodical release, which may counteract such conditions; and the facts clearly indicate that a leading part in this process is played by a nuclear reorganization effected by maturation, by syngamy or by both combined. And such results as those observed in *Uroleptus* indicate that the same is true in some of the Protista even if not in all.

2. Increase of Vigor by Heterosis (Heterozygosis) ¹

The increase of size or vigor or both in crosses between different races, varieties or species long since attracted the attention of horticulturists, breeders of domesticated animals and students of heredity. Darwin's admirable researches on the beneficial effects of cross-fertilization in higher plants directed general attention to the subject. More recently it has been carefully reëxamined from the standpoint of the Mendelian analysis by East, Shull, Emerson, Jones, and others who have strikingly demonstrated the remarkably favorable effects produced by crossing different strains of maize and other plants; and Whitney ('12) has produced evidence in the same direction in the case of rotifers. The earlier results in this field gave rise to a rather widespread belief that crossing is *per se* a cause of increased vigor and that inbreeding is in the same sense a cause of decreased vigor or degeneracy. Darwin seems on the whole to have held to this view

¹ For recent general discussions of this subject, see Shull ('10), East and Hayes ('12), Jones ('17).

of inbreeding, though he was well aware of the difficulties offered by plants such as Leguminosæ, that are habitually self-fertilizing. On the other hand, he showed that it is not the mere crossing of any two individuals which is beneficial to the offspring; for it does not appear, or is very slight, in plants of the same stock intercrossed during several generations.¹

From this and much other evidence of the same type Darwin concluded that the benefit results from some "difference of constitution between the sexual elements," or, in more modern terms, a difference of transmissible factors in the gametes; and this is fully borne out by the recent Mendelian analysis of the problem. The precise nature of these factors is still a subject of discussion.² The increased vigor of hybrids has been cited by some writers in support of the theory of rejuvenescence; but the argument undoubtedly loses much of its weight in view of the facts that have been outlined, and it is wholly rejected by East and Hayes.³ It may be added that since the factors which determine increase of vigor in heterozygosis are transmissible they are probably borne by the chromosomes; but the evidence is conclusive that the initiation of development is not in general due to the nuclei or their union, but to other factors (p. 395). Hybrid vigor may therefore be a phenomenon wholly different from the stimulus to development given to the egg by the sperm, or effected by conjugation in the Protista. Nevertheless the suspicion cannot be avoided that a key to rejuvenescence (if we may so designate the activation of the egg by the sperm and its consequences in higher organisms) may yet be found in the study of heterosis.

3. Syngamy, Heredity and Variation

In dioecious organisms syngamy obviously brings together two originally separated lines of heredity and thus effects *amphimixis* (Weismann). It is less obvious, but probably equally true, that new combinations of hereditary traits may also result from autogamy or self-fertilization and the associated processes of maturation, as will later appear (p. 950). As above indicated (p. 233) sexual reproduction often yields a more varied progeny than asexual; and out of this fact grew the conclusion that syngamy is a source of variation—a view long since suggested by Treviranus and later developed by Darwin, Spencer, Brooks ('83), Weismann ('91) and many later writers, including some of the most recent experimenters. In case of Protista, Jennings ('11-'13) has made an elaborate experimental study of the phenomena in *Paramœcium* the results of which, as already indicated, are quite in harmony with the conclusions of Weismann. "Conjugation

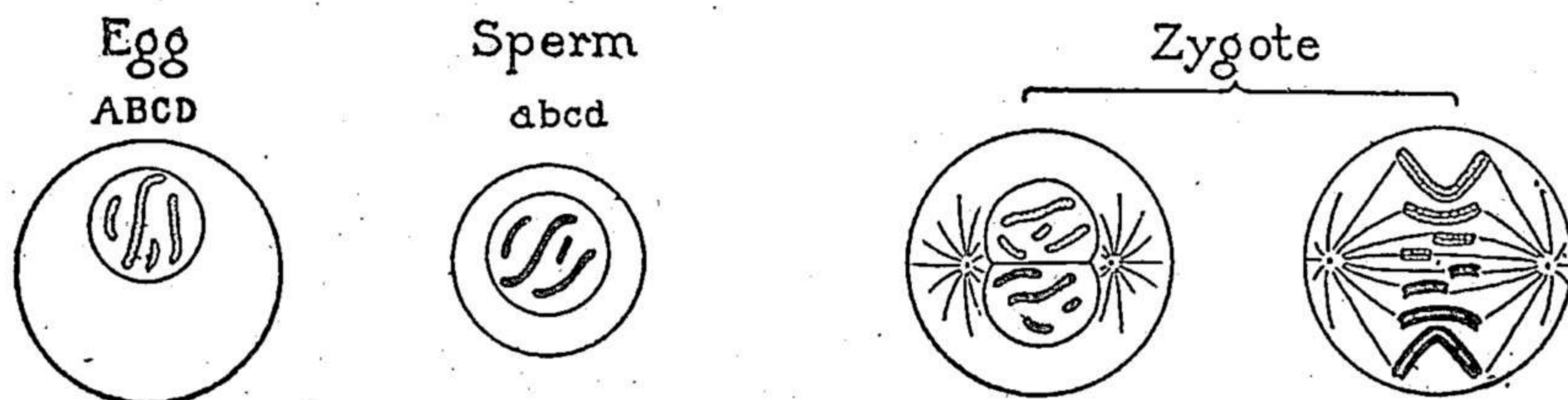
¹ *Effects of Cross-and Self-Fertilization*, p. 269.

³ *Op. cit.*, p. 38. See also Shull.

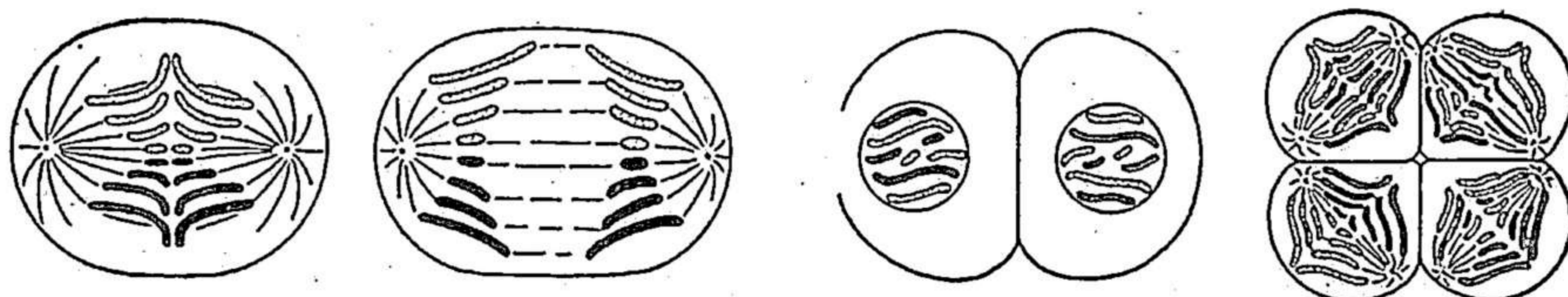
² Cf. Jones ('17).

produces within a pure race heritable differentiations; so that as a result races diverse in their heritable characters arise from a simple race with uniform heritable characters. . . . What conjugation does is to bring about

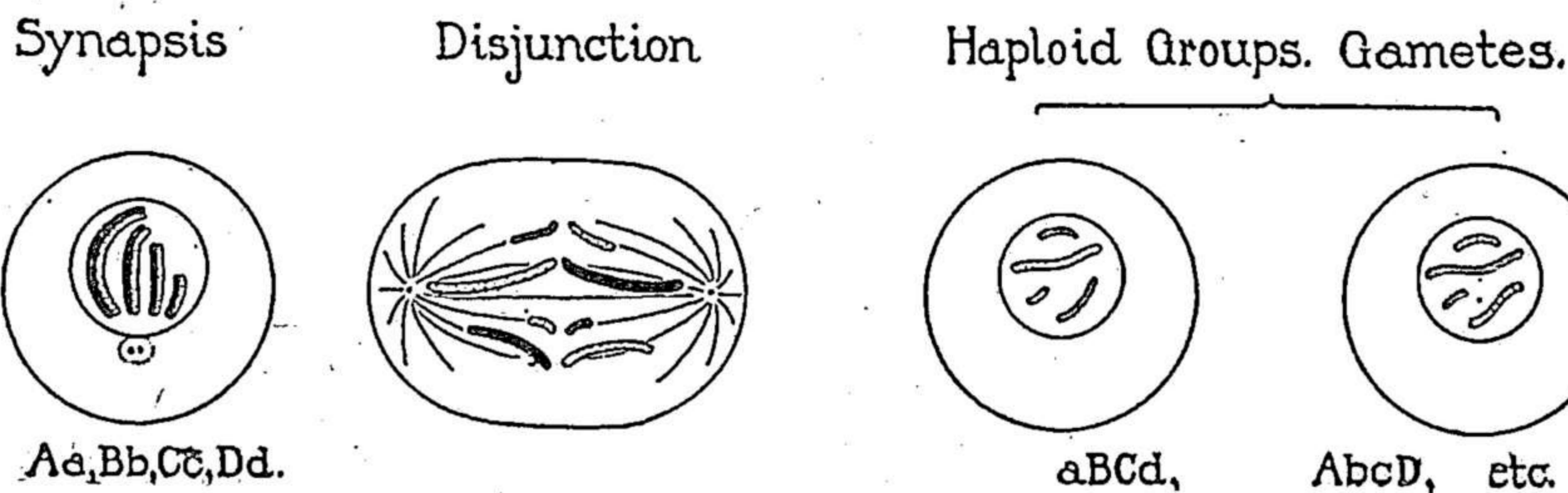
Union of the Haploid Groups. Fertilization.



Division of the Diploid Group. Mitosis.



Reduction of the Diploid Groups to Haploid. Meiosis.



Recombinations in Fertilization.

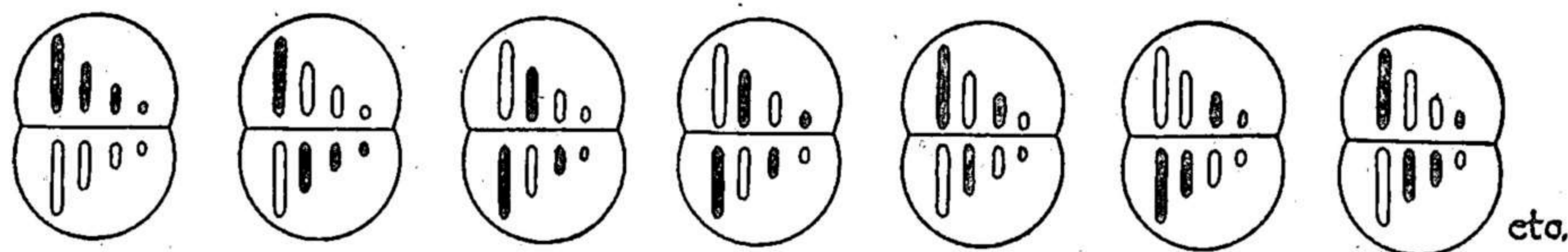


Fig. 102.—General diagram of the chromosome-cycle in animals, the haploid groups assumed to contain four chromosomes of different sizes. Paternal and maternal chromosomes in white and black respectively. Complete gonometry of the diploid groups in the initial stages, later lost. Only two of the 16 possible chromosome-combinations in the gametes shown (cf. Fig. 105), and only seven zygote-combinations out of 256 possible ones.

new combinations of germ-plasm, just as is done in the sexual reproduction of higher animals. One result of this is to produce biparental inheritance; another is to give origin to many variations, in the sense of inherited differentiations between different strains. Some of the new combinations are

better adapted to the existing conditions than others; these survive while the others die out" ('13, p. 378). On its face this seems to be essentially the same conclusion as that of Weismann that amphimixis produces variations—or rather assures their “mingling and persistent renewal”—thus giving the material on which natural selection operates. Jennings, however, here has in mind the modern Mendelian analysis, which has proved that the diversity arising from syngamy (*e. g.*, in the sexual reproduction of cultivated races of plants) is largely due to heterozygosis and recombination, *i. e.*, to the separation and reunion in new groupings of definite heredity factors that have previously been separated in different individuals, strains or races. What is new in such cases, is not the genes or factors concerned but only their particular modes of combination. It is, therefore, a debatable question whether syngamy can produce new variations in the sense that this word was employed by Weismann and the earlier writers; and the origin of really new elements or factors out of which new characters may be built up constitutes one of the main problems now before students of genetics.

Lastly it may be added that a number of writers, even including some of those who have recognized syngamy as a source of variations, have held that syngamy also serves to maintain the stability of species through intercrossing. This view, which appears in Darwin, has been urged by O. Herting, Strasburger, Quetelet, Galton, and others, and finds expression in Galton's so-called law of regression.

4. Syngamy, Meiosis and the Cycle of the Chromosomes

In turning, lastly, to the cytological aspects of syngamy and its associated phenomena we find ourselves on firmer ground. Our most definite knowledge in this field relates to the history of the nuclei and the cycle of the chromosomes, which alone will be considered at this point. Syngamy, it is true, often involves a union of gamete-protoplasms (*plastogamy*) as well as of nuclei (*karyogamy*); but this aspect of the phenomena is still too little known to be profitably taken up here.

Concerning the history of the chromosomes, we are now able to state three principal conclusions, each of fundamental importance for the problems of heredity. Two of these are in principle well established for many higher plants and animals, while the third is hardly less certain. They are essentially as follows:

a. Karyogamy and Establishment of the Diploid Chromosome-Groups. In the course of syngamy the two gamete-nuclei unite or become closely associated (*karyogamy*) each giving rise meanwhile to a single or haploid group of chromosomes the number of which is half the typical somatic

number of the species ("Van Beneden's Law"). The two groups thus associated in the zygote, respectively of maternal and paternal origin, are nearly alike in respect to both the number and the size-relations of the chromosomes (Figs. 103, 104) and are in other respects nearly equivalent. The double or *diploid* group thus produced is perpetuated throughout the life of the resulting individual, being handed on by progressive division to all the nuclei of the body without change in its essential character. Certain

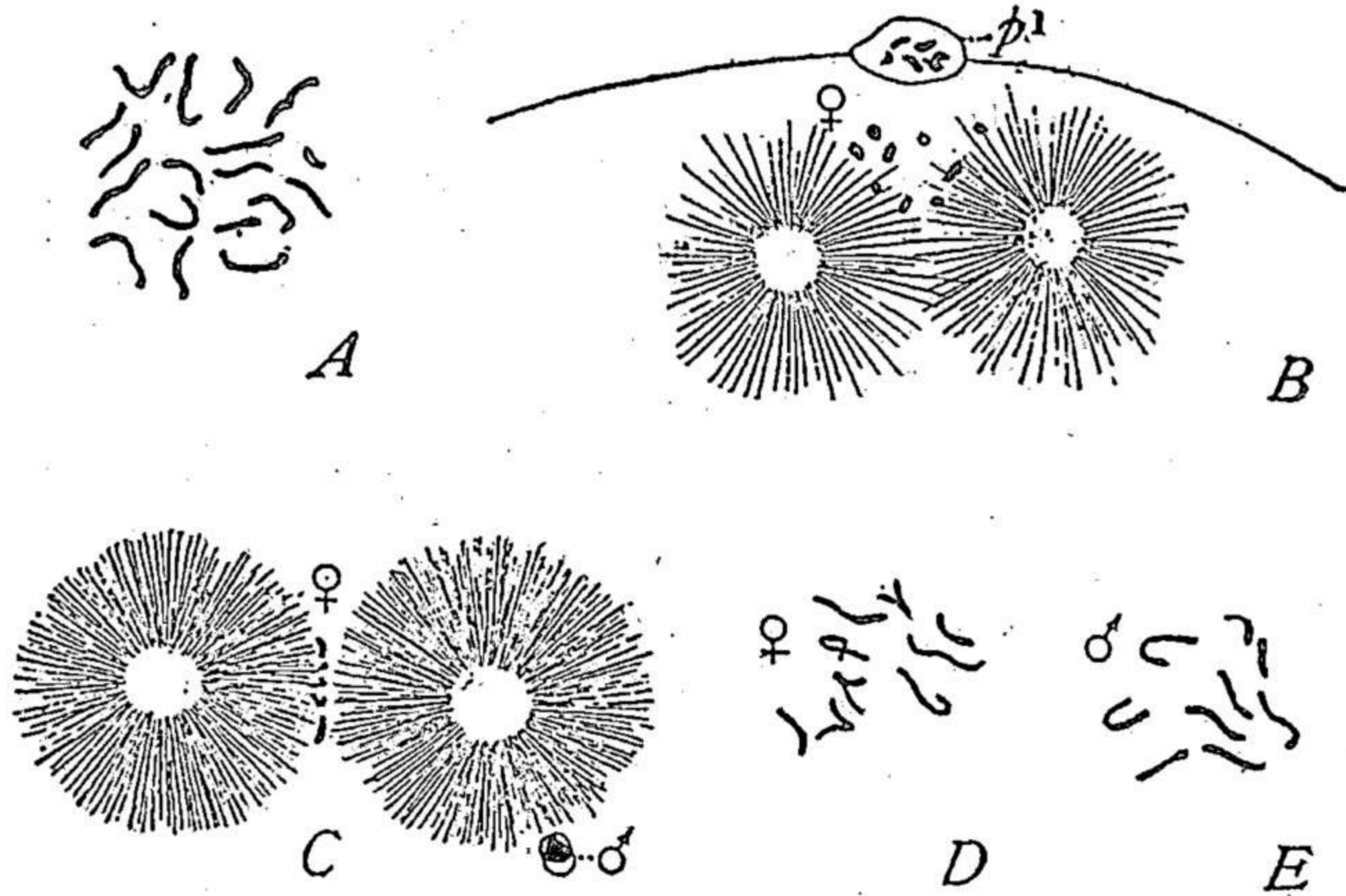


Fig. 103.—Diploid and haploid chromosome-groups in the sea-urchin *Echinus microtuberculatus* (BOVERI).

A, diploid group, 18 chromosomes, polar view of first cleavage of the fertilized egg; B, prophase of second polar spindle, haploid group, 9 chromosomes; C, partial fertilization, sperm-nucleus at ♂; D, polar view of same, 9 chromosomes; E, polar view of metaphase-group from sperm-amphiaser in merogony (first cleavage), 9 chromosomes.

deviations from the typical phenomena as just outlined will later be described (pp. 75, 841).

b. *Meiosis. Reduction of the Diploid Groups to Haploid.* At a certain period during the life-cycle the number of chromosomes is again reduced to one-half; *i. e.*, the double or diploid chromosome-groups are reduced to single or haploid ones (Fig. 102). These numbers and other characteristics of the chromosome-groups are thus held constant from generation to generation. This process, known as *reduction* or *meiosis*, belongs among the general phenomena of *maturation*, which will form the subject of Chapter VI. At this point we indicate only certain of its most general features.

The period at which meiosis takes place varies widely in different cases (p. 488). In animals generally it constitutes one of the steps in the formation of the gametes themselves. In the male it takes place in the testis during the formation of the sperms. In the female it is delayed until after the egg has attained its growth often until after it has left the ovary, or even until after the sperm has entered, meiosis then being intimately associated with fertilization (p. 398). In most plants, on the other hand,

meiosis has no immediate relation either to fertilization or to gamete-formation, but takes place long before, during the process of spore-formation. In a third and much rarer case meiosis takes place in the zygote immediately after karyogamy, so that the developing zygote and all of its products are of haploid constitution. This is known only in certain algæ and a few other

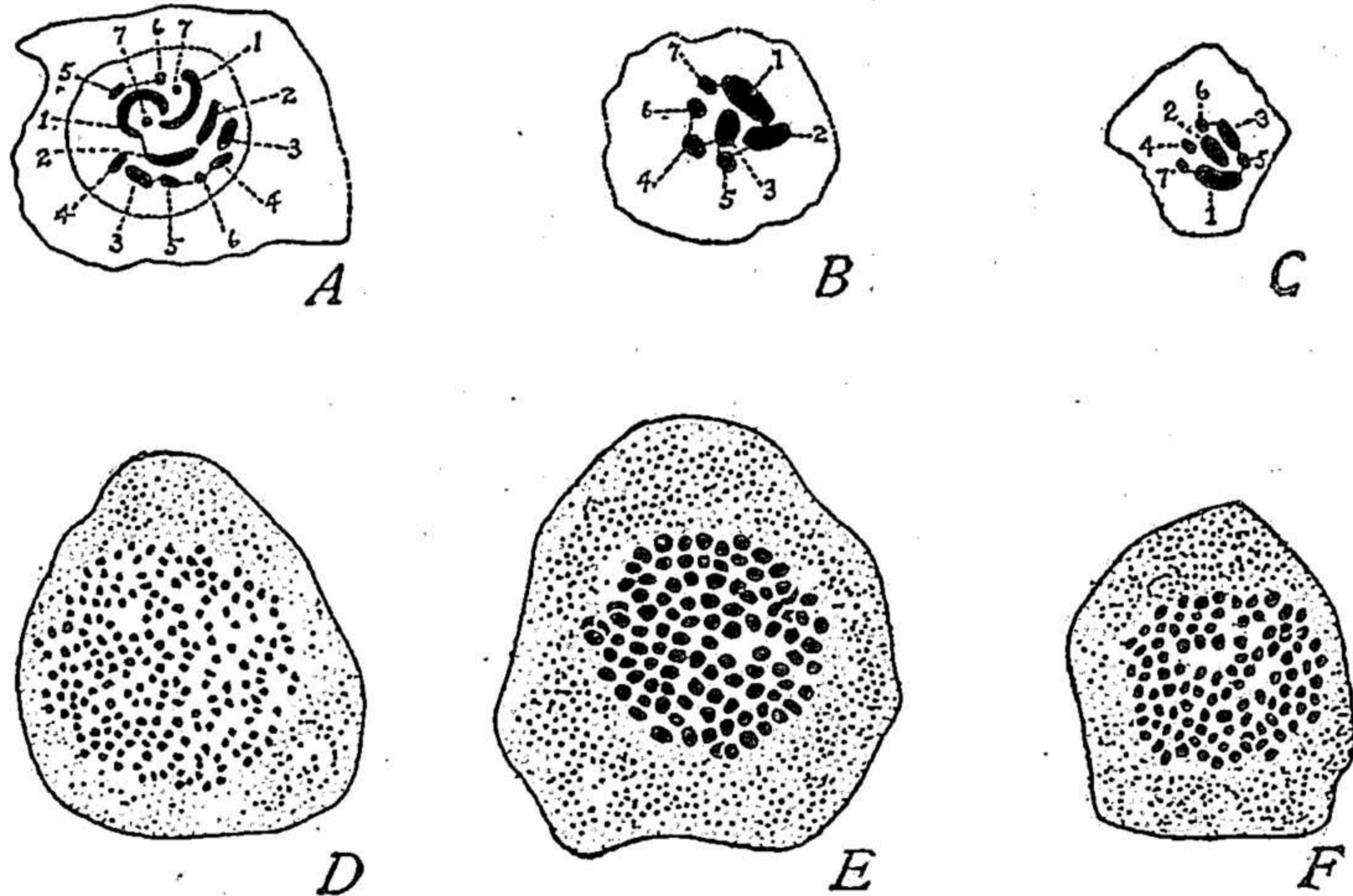


Fig. 104.—Diploid and haploid chromosome-groups, A-C in the aphid *Aphis rosæ* (STEVENS), D-F in the crayfish *Cambarus virilis* (FASTEN).

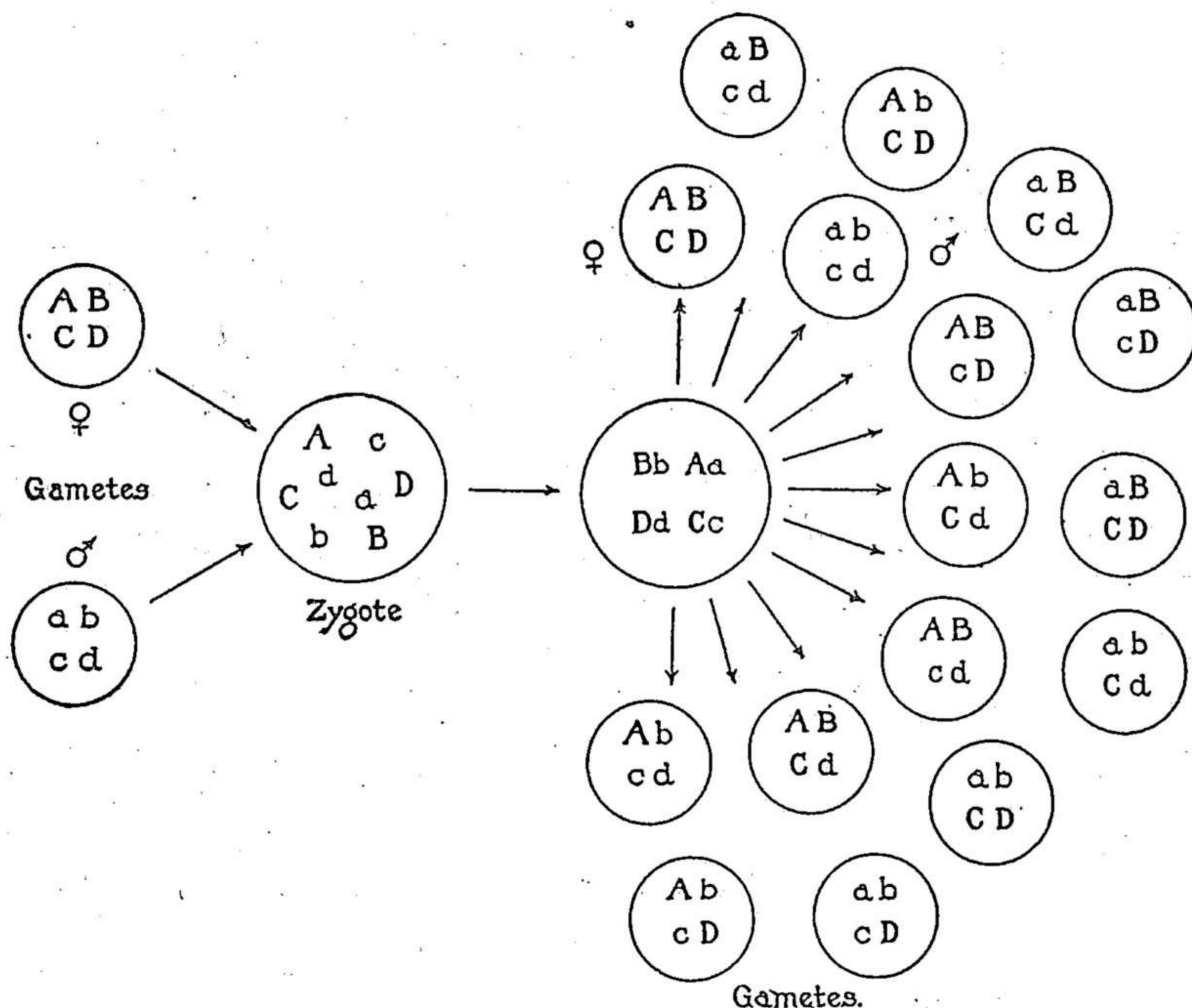
A, diploid group, 14-chromosomes, metaphase of second cleavage of a parthenogenetic egg showing size-differences and chromosome-pairs; B, pseudo-haploid group from metaphase of first spermatocyte-division, 7 bivalent chromosomes; C, true haploid group, second spermatocyte-division, 7 single or univalent chromosomes.

D-F, corresponding series in a species having numerous chromosomes with only slight size differences. D, diploid group, 200 chromosomes, from spermatogonial division; E, pseudo-haploid group, metaphase of first spermatocyte 100 bivalents; F, true haploid group, second spermatocyte, 100 univalent chromosomes.

simple forms. In all of these cases the final result is the same; the gamete-nuclei receive a single or haploid group of chromosomes.

c. *Segregation, Regrouping and Random Assortment of the Chromosomes.* Reduction is brought about by a modified form of mitosis in which the chromosomes undergo a regrouping or segregation, the diploid group separating into two haploid groups without undergoing division in the usual sense. In the course of this process, as will later be shown (Chap. XII), is also effected a regrouping of the original paternal and maternal chromosomes (*i. e.*, of their individual descendants), such that all possible recombinations of them are delivered to the resulting gametes, in so far as this does not alter the integrity of the group as such. This will be made clear from the diagram (Fig. 105). If, for example, the number of chromosomes be assumed to be four, the original maternal haploid group may be designated as *A B C D*, and the paternal group as *a b c d*, and the zygote receives the diploid group *ABDC abcd*. In maturation every gamete receives a group of

four chromosomes, corresponding to the original groups, but any particular member of the group may be of either maternal or paternal origin. If this distribution follows the law of chance, 16 such combinations should result,



Recombinations with Independent Assortment.

Fig. 105.—Diagram applicable either to chromosomes or to hereditary factors, to show Mendelian segregation and independent assortment. (Linkage phenomena are here left out of account.) Maternal components (chromosomes or factors) in capitals, paternal in small letters. The haploid number assumed to be four (*cf.* Fig. 102).

in equal numbers, of which only two ($ABCD$ and $abcd$) are identical with the original ones. The same rule holds with larger numbers.

d. Chromosomes and Mendelian Heredity. In these facts, as will be set forth in Chapter XII, lies the explanation of the Mendelian segregation of the factors of heredity. The history of the chromosomes in the life-cycle runs exactly parallel to that of these factors. Like the chromosomes, the factors or Mendelian units are single or haploid in the gametes, double or diploid in the zygote, and its products; their redistribution and recombination in the gametes follow precisely the same course as that of the chromosomes.¹ The critical study of these facts and a multitude of others has removed all doubt that the chromosomes play an essential rôle in heredity.

We now proceed to a more detailed examination of the phenomena by an examination of the structure and origin of the germ-cells (Chap. IV), their

¹ The phenomena of linkage are here left out of account.

union in fertilization (Chap. V), and their history in maturation and reduction (Chap VI).

LITERATURE III

(See also V, VII, XII. For abbreviations see General Literature List).

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