The History and Control of Sex.

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The student of sex and closely related problems of heredity may rationally ask himself any or all of the following questions: What is the significance of sex? or, in other words, why are organisms male and female? Is the sex of the organism determined during the early development of the individual? or is it predetermined in the germ cells? If the former, what conditions of the environment are favorable to the development of males and what to females? If the latter, what is it in the gametes or sex-cells that predetermines maleness or femaleness?

As in the establishment of the doctrine of sexuality itself, these questions can be answered by experiment only and by the microscopic investigation of the germ cells and the manner of their development. As an introduction to what I shall have to say in this paper concerning sex control, I desire to point out briefly those lines of study which seem to me to have been most effective in establishing the doctrine of sexuality in plants; for it will be seen that the lines of investigation which established the theory of sex are similar to those that are yielding the most fruitful results in the study of the more difficult hereditary problems of the present day.

When in the history of civilized or semi-civilized man, the idea arose that plants possess sex, no one can tell or perhaps imagine. Before the days of written history the old Arabs of the desert knew that certain palm trees produced fruit, while others did not, and, in order that the fruit might develop abundantly, it was necessary to bring the flowers of the sterile trees and hang them upon the branches of those which bore the fruit. It is evident that they also practiced the caprification of the fig, using the same methods employed at the present time in the fig-growing localities along the Mediterranean, for we read in Herodotus who, in speaking of the Babylonians, states that, "The natives tie the fruit of the male palms, as they are called by the Greeks, to the branches of the date-bearing palm, to let the gall-fly enter the dates and ripen them, and to prevent the fruit falling off. The male palms, like the wild fig trees, have usually the gall-fly in their fruit." Herodotus was in error in regard to the presence of the gall fly in the palm, and it is said that Theophrastus was the first to point out the inaccuracy in the statement. This brilliant and gifted pupil of Aristotle was probably the foremost of all ancient botanists, for, it is said, he knew six hundred plants. The ideas of Theophrastus upon this subject seemed to be more definite than those of his great teacher. He regards the palm and terebinths as being some male and some female, for "it is certain," he says, "that among plants of the same species some produce flowers and some do not; male palms, for instance, bear flowers, the female only fruit." Let it be borne in mind here that neither Theophrastus nor the botanists of the 16th and 17th centuries considered the rudiment of the fruit to be a part of the flower. Theophrastus probably added very little to the knowledge of sexuality in plants which had been handed down to him either in the form of tradition or through the scanty writings upon natural history. That he seemed to have made no observations upon the subject, but to have relied in a large measure upon heresay, is apparent from the following: "What men say that the fruit of the female date-palm does not perfect itself unless the blossom of the male with its dust is shaken over it, is indeed wonderful, but it resembles the caprification of the fig, and it might almost be concluded that the female plant is not by itself sufficient for the perfecting of the foetus." In the time of Pliny, this idea of sexual difference in plants had been pretty well confirmed in the minds of educated men. In his "Historia Mundi," in describing the relation between the male and female date-palm, Pliny calls the pollen-dust the material of fertilization, and says that naturalists tell us that all trees and even herbs have the two sexes.

Now while the ancients had some notion of sex in plants, their ideas were based chiefly upon certain apparent analogies with animals. It must be borne in mind that whilst the ancients attributed to the pollen the power of fertilization, they had no notion that this fertilization was anything further than some unexplained subtile influence of the flower dust upon the fruit. However, we should wonder only at how much they knew in the days of Herodotus and Theophrastus as compared with the progress of knowledge made along this line during the following two thousand years: for the time from Aristotle to the discovery of the cell by Robert Hooke, the publication of the great works on anatomy by Malpighi and Grew, and the experiments of Camerarius in the latter part of the 17th century, was a lapse of long and dreary centuries in the history of science.

This was not because there were no men willing to devote their time to natural history, but chiefly because of the attitude of mind which demanded that problems arising be not solved by observation and experiment, but by the process of deductions from the authorities. The question was not, what do the observed facts teach? but, how are they to be interpreted from what Aristotle says?

The improvement of the microscope and the extensive studies on the minute anatomy of plants did not bring the results that might have been reasonably expected. In spite of his excellent work on the anatomy of plants, Grew seemed to have been unable to gain any true insight into the structure and function of pollen. He did not even consider the stamens as the so-called male members of the flower, speaking of them only as the attire, but he records a conversation with an otherwise unknown botanist. Sir Thomas Millington, who was probably the first person to claim for the stamens the character of male organs. I quote from the "Anatomy of Plants" (chap. V, secs. 3 and 4, page 171) : "In discourse hereof with our learned Savilian professor, Sir Thomas Millington, he told me he conceived that the attire doth serve as the male for the generation of the seed. I immediately replied that I was of the same opinion and gave him some reasons for it and answered some objections which might oppose them." But how badly Grew must have been confused in the matter may be seen from his description of the florets in the head of certain Compositae. He regarded the style and stigma of the floral attire as a portion of the male organ, speaking of the small globulets (pollen grains) in the thecae (anthers) of the seedlike attire as a vegetable sperm which falls upon the seed case and so "touches it with a prolific virtue." Grew could conceive of sex in plants only in the form of certain apparent analogies with animals. He reasoned that the same plant may be both male and female. because snails and some other animals are so constituted, but to complete the similarity between the plant and the animal would require that the plant should not only resemble the animal, but should actually be one. Down to the year 1691, about all that was known concerning the sexuality in plants was comprised in the facts related by Theophrastus for the date-palm and the terebinth, and in the conjectures of Millington, Grew and others, while Malpighi's views in opposition to these authors were considered equally well founded.

The doctrine of sexuality in plants could only be raised to the rank of scientific fact by experiment. It was necessary to show that no seed capable of germination could be formed without the aid of pollen, and all historic records concur in proving that Rudolph Jacob Camerarius was the first to attempt to solve the problem in this way. Dioecious plants were cultivated apart from each other, but no perfect seeds were formed. He removed the stamens from the flowers of the castor oil plant and the stigmas from maize, with the result that no seeds were set in the castor oil plant, and in the place of grains of corn only empty husks were to be seen. The results of Camerarius were published in 1691-94. At this time the authority of the ancients was so great that Camerarius thought it necessary to insist that the views of Aristotle and Theophrastus were not opposed to the sexual theory. Among the few experiments carried out in the next fifty years were those of the Governor of Pennsylvania, James Logan, an Irishman by birth. Logan experimented with some plants of maize. Upon a cob from which he removed some of the stigmas, or silks, he found as many grains as there were stigmas remaining. One cob which was wrapped in muslin before the silks appeared, produced no kernels. In 1751, Gleditsch, director of the botanic garden in Berlin, had been told that a date palm eighty years old, which had been brought from Africa, never bore fruit. As there was no staminate tree of the species in Berlin, Gleditsch ordered pollen sent from Leipzig. The journey required nine days, and although Gleditsch thought the pollen spoiled, the male inflorescence was hung upon the Berlin tree, with the result that seeds were set which germinated in the following spring.

The century following the discovery of Camerarius was characterized by two lines of investigation which, more than any other activity of botanists, led to the complete establishment of the sexual theory. I refer to the refutation of the old theory of evolution together with the birth of the doctrine of epigenesis, and the discovery of hybridization; the first of these being the outcome of microscopic studies, and the latter that of experimentation. It may be said in this connection that the history of biological science teaches that the greatest and the most substantial progress has been made where the studies of the morphologist and of the experimenter have gone on side by side, the one serving as a control upon the other. According to the old theory of evolution, or the inclusion theory, that the germ in every seed, for example, contained all the parts of the organism, and that this germ enclosed a similar one in miniature, and so on, like a box within a box. This view of the inclusion of germ within germ was very prevalent in the 18th century, and Kaspar Friedrich Wolf (1759) has been given the credit of refuting it. Wolf, in his doctor's thesis on the "Theory of Generation," maintained that the embryo and organs of a plant develop not by the unfolding of parts already present in miniature, but that they grew out of undifferentiated rudiments, the theory of epigenesis. However, Wolf's arguments were far from convincing, as he held that the act of fertilization was merely another form of nutrition.

About the same time experiments in hybridization were being carried on by several investigators, and the results obtained supplied much more convincing proof against the old theory of evolution. Among the foremost men in this field were Gottlieb Koelreuter and Christian Konrad Sprengel. While Kolreuter brought together many important observations on the sexuality of plants, yet his greatest service consisted in the production of hybrids. In this connection it may be of interest to note that his first hybrids were produced between two species of tobacco plants, *Nicotiana panicum* and *N. rustica*. What he accomplished did not require being changed, but when combined with later observations has been used in the discovery of general principles of hybridization. His work seems to belong to our time. Koelreuter showed that only closely allied plants, and not always these, were capable of producing hybrids, and that the mingling of parental characters in the hybrid was the best refutation of the theory of evolution. It was no easy matter to place the proper estimate upon the value of the contributions of this gifted observer. The collectors of the Linnaean school, as well as the true systematists at the close of the 18th century, who wielded a powerful influence upon botanical thought, had little understanding for such labors as Koelreuter's, and incorrect ideas of hybrids prevailed in spite of botanical literature. Hybrids were also inconvenient for the believers in the constancy of species.

Koelreuter's studies were not confined to hybridization alone, for he directs attention to the natural way of the transfer of pollen from stamen to stigma, being the first to recognize the agency of insects. He studied pollen grains, showing that fertilization followed pollenation in the absence of light, and rejected the idea that the pollen grain passed bodily into the ovary. With the microscope, however, he was less skillful than as an experimenter, for he supposed the pollen grain to be solid tissue, and the fertilizing substance to be oil which adheres to the outside of the grain and finds its way to the ovule. The pollen tube had not been discovered, although the time was one hundred years after the discovery of the cell by Robert Hooke.

As Camerarius first proved the sexuality of plants, and Koelreuter showed that different species can unite sexually to produce hybrids, so Sprengel demonstrated that a certain kind of hybridization was very common in the vegetable kingdom, namely the crossing of flowers of different individuals of the same species. To him belongs the credit for having first shown the part played by insects in cross pollenation, and pointing out the correlation between such properties of the flower as color, odor, nectar, special forms and markings, and so forth, and the visiting insects.

Karl Friederick Gaertner, son of Joseph Gaertner, took up the work so ably begun by Koelreuter, and greatly extended the knowledge of hybridization, having kept accurate account of nine thousand experiments. His work was published in 1849. Sachs states that "These observations once more confirmed the existence of the sexuality in plants, and in such a manner that it could never again be disputed. When facts were observed in 1860, which led to the presumption that under certain circumstances in certain individuals of some species of plants, the female organs might produce embryos capable ot development without the help of the male, there was no thought of using these cases of supposed parthenogenesis to disprove the existence of sexuality as the general rule; men were concerned only to verify first of all the occurrence of the phenomena, and then to see how they were to be reasonably understood side by side with the existing ideas of sexuality." Gaertner's experiments were conducted at Claw, in Wurtemberg, the place in which Koelreuter carried on his studies; Camerarius worked in Tübingen.

While the experimenters in hybridization were at work, the student with the microscope was no less busy. In 1823, Amici discovered the pollen tube in the stigma, and the fact was confirmed by others. In 1830, the same observer traced the pollen tube into the ovule. Schleiden and Schacht now came forward with their erroneous theory of the formation of the embyro in the seed. They maintained that the embyro develops from the end of the pollen tube after the latter enters the ovule. It is clear that this doctrine would do away with the essential point in the sexuality of plants, for the ovule would be regarded merely as an incubator for the embyro. Amici, in 1846, brought forth decisive proof for the view he had maintained, namely, that the embryo arises not from the end of the pollen tube, but from a portion of the ovule which already existed before fertilization, and that this part is fertilized by a fluid contained in the pollen tube. The correctness of this view was confirmed the following year by von Mohl and Hofmeister, the latter of whom described the points in detail which decided the question, and illustrated them with beautiful figures.

Following the publication of Amici, a vehement controversy arose between the adherents of the views of Schleiden and those of Amici. A prize offered by the Institute of the Netherlands at Amsterdam was awarded to an essay by Schacht in 1850, which defended Schleiden's theory, and illustrated it by a number of drawings giving both incorrect and inconceivable representations of the decisive points. In this case the prize essay was refuted before it appeared, by von Mohl, Hofmeister and Tulasne. Von Mohl's words uttered in 1863 in regard to the value of prize essays are so fitting at the present day that I can not refrain from quoting. He said: "Now that we know that Schleiden's doctrine was an illusion, it is instructive, but at the same time sad, to see how ready men were to accept the false for the true; some, renouncing all observation of their own, dressed up the phantom in theoretical principles; others with the microscope in hand, but led astray by their preconceptions, believed that they saw what they could not have seen, and endeavored to exhibit the correctness of Schleiden's notions as raised above all doubt by the aid of hundreds of figures, which had everything but truth to recommend them; and how an academy by rewarding such work gave fresh confirmation to an experience which had been repeatedly made good especially in our own subject during many years past, namely, that prize essays are little adapted to contribute to the solution of a doubtful question in science."

The discovery of the sexual process in cryptogams by Thuret, Pringsheim and others followed within four or five years after the complete establishment of that process in the higher plants. It seems strange to us now that a phenomenon so easy of observation was not discovered until its occurrence had been completely demonstrated in organisms presenting the greatest difficulties to its investigation. However, it is of interest to recall that just thirty-two years ago Strasburger traced the essential constituents of the nucleus in unbroken sequence from one cell generation to another, thus establishing for the nucleus the rank of morphological unity; and just thirty-two years ago also Oscar Hertwig showed that fertilization consists essentially in the union of the two gamete nuclei. It only remained now for later studies on the cell to confirm and to establish the doctrine that the nucleus is the bearer of the heredity characters.

With this is view, we are now ready to consider some of the modern phases of our subject.

Any effort to trace the development of the sexual process with all correlated phenomena would lead us into an overwhelming mass of details. Consequently, I shall merely recall that among the lowest plants sexuality does not exist, and that, in the simplest plants with a sexual process, the sex cells, or gametes, are scarcely to be distinguished from the non-sexual reproductive cells. The conclusion is that gametes were originally derived from a sexual propagative cells. There is accordingly no differentiation into male and female. The life cycle of these simple sexual plants is also simple, and it is reasonable to suppose that a corresponding degree of differentiation obtains in the chromatin or hereditary substances of the sex cells. As we ascend in the scale of evolution toward higher and more complex organisms, we find a corresponding differentiation in all structures and functions, and may we not assume also that the hereditary substance, or germ plasm, is likewise specialized and differentiated? Therefore, sex is the expression of a very fundamental sort of division of labor. I do not mean by that a division of labor which is of a secondary nature such as man has ascribed to the individuals of his own species, but that of a purely hereditary character—or may I say maleness and femaleness in the broadest and most fundamental sense.

How then did sex come about? And what is it that determines that one individual or member of a life cycle will be male and another female? To ask such questions fifty or even twenty-five years ago might have been regarded as visionary. Not so today. Considered from the botanical standpoint, the problem of sex determination has to deal with a certain category of phenomena that are in many respects fundamentally different from those presented by animals. In plants in which sex differentiation is well defined, there is in every complete life cycle two phases known as the sexual and the asexual, or gametophyte and sporophyte. The sporophyte springs from the fertilized egg or the union of sex cells. This sporophyte in turn bears spores which give rise to gametophytes. This may be made clear by means of an example such as the fern. The spores borne on the leaves of the fern do not produce directly new ferns, but very small plants known as prothallia, which in the simpler ferns are independent and self-nourishing individuals. The prothallia are the sexual plants. They bear the sex organs, which is turn produce eggs and sperms. The prothallia may be either purely male or female or hermaphrodite. When the egg is fertilized it develops immediately into the sporophyte, or what we commonly know as the fern. Thus the sexual plant, or gametophyte (female gametophyte) not only produces the sex organs, but serves as the incubator and brooder for the young sporophyte. The life-cycle of the highest plants such as trees and sunflowers consists also of these two generations, but the relative size and mutual relation of sporophyte and gametophyte are different in the higher plants. For example, the beech tree is the sporophyte, the gametophyte being the pollen tube and the embryosac of the undeveloped seed. Here the reverse condition prevails as regards the mutual relation of sporophyte and gametophyte to that in the fern, namely, the sporophyte nourishes the young sporophyte as well as both gametophytes.

Now, we are in the habit of speaking of male and female flowers according as they are wholly staminate or pistillate, and the plant that bears only staminate flowers we call male, while the one bearing only pistillate flowers is designated as the female individual. However, in the strict morphological sense the sporophyte is without sex, hence trees can be neither male nor female, and to avoid trouble and useless discussion, it is doubtless better to speak of staminate and pistillate trees; for we shall see that the sex of any complete life-cycle is determined and fixed in the germ cells. From the foregoing it is quite clear that in the animal kingdom, apart from one or two cell generations, there is nothing in the life-history that is comparable to sporophyte and gametophyte.

We are now ready to answer the question, upon what does the differentiation into gametophyte and sporophyte depend? Our explanation of this doctrine is based upon the theory of the hereditary substance. Doubtless nearly all biologists concur in the view that the hereditary characters are borne by a substance in the nucleus of the cell called chromatin. When the nucleus divides the chromatin differentiates into a definite number of pieces known as chromosomes. The number of chromosomes is always constant for the reproductive cells of any species. In all the cells of the sporophyte of any plant, which lie in the germ tract, there are, let us say, a definite number of chromosomes designated by n. During the formation of spores, however, the number is reduced to one-half, or n_2. Now each spore has n_2 chromosomes, and the cells of the gametophyte resulting therefrom will possess n_2 chromosomes; consequently the egg and the sperm will have each n_2 chromosomes. It is apparent that when egg and sperm unite, the fecundated egg and the individual arising from it will contain n_2 plus n_2 or n chromosomes.

The most fundamental difference between sporophyte and gametophyte lies in the fact that the latter possess just one-half as many chromosomes as the former. This hereditary difference between sporophyte and gametophyte and the change which brings about the transition may be made clear by means of the following figure, showing diagramatically the behavior of the chromatin. Fig. 1 illustrates the behavior of the chromatin in an ordinary vegetative cell. Here the chromatin segments passing into the new nuclei are formed by a longitudinal fission of a single chromosome—an equational division. In Fig. 2, *a* to *f*, is shown the first or reducing division in spore mother cells. One-half of the somatic chromosomes pass to one of the daughter nuclei and the other half to the other, thus bringing about the reduction of the number. The second division in the spore mother cell (*g* to *i*) is equational.

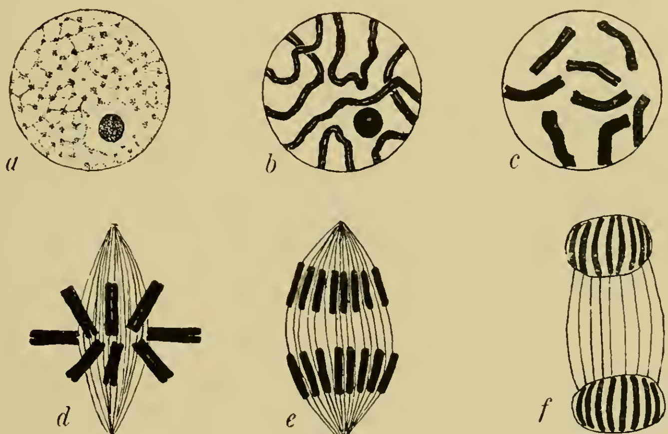

Fig. 1.

Fig. 1. Diagrams showing the behavior of the chromatin during an ordinary somatic mitosis. *a.* nucleus in resting condition, showing chromatin distributed in small granules within the linin network and a nucleolus. *b.* the chromatin spirem has formed and it has split longitudinally. *c.* the spirem has segmented into chromosomes, e. g., eight. *d.* spindle stage; chromosomes arranged in the equatorial plate. *e.* anaphase; daughter chromosomes moving toward the poles of the spindle. *f.* daughter nuclei, each containing eight daughter chromosomes. Such a division is known as equational, since the two resulting nuclei are hereditarily alike.

The parallel between plants and animals is found in the phenomenon of the reduced number of chromosomes in the sex-cells, with this distinction, that in higher plants the reduction in the number of the chromosomes occurs when the spores are formed, which may be many thousands of cell generations removed from the time in ontogeny when eggs and sperms are differentiated; while in animals the reduction immediately precedes the formation of the gametes. In regard to the chromosomes themselves, the view generally prevailing is that each possess a distinct identity or individuality which is maintained throughout ontogeny, and phenomena pertaining thereto have been presented under the theory of the individuality of the chromosomes. Very recently, however, the idea of individuality has been taken away from the chromosomes and applied to smaller units, such as the chromomeres, or better the microscopically distinguishable granules which make up the chromomeres. We may call these particles pangens, or select any name which may be convenient and likely of adoption. The writer has expressed his views on this subject in greater detail in a recent publication, and only a few brief statements will be made here, in as much as a fuller discussion is regarded as being too technical for a general audience. The idea of individuality is applied to the chromomeres or the small particles composing them, chiefly because the identity of the chromosome is lost in the restin nucleus. There are no good reasons to believe that a given chromosome always contains the same hereditary qualities in any succession of cell generations. Furthermore, no special importance should be attached to the different sizes of the chromosomes, for, as a rule, one of the most striking phenomena in a dividing nucleus is the marked difference in the size of the chromosomes. These small material particles, or pangens, are responsible for the characters of the individual, although they are not regarded as the immediate characters themselves. They may be roughly compared with ferments, bringing about changes which collectively constitute development, and produce those chemical re-arrangements of which form, color, and so forth, are the visible expression. Fused gamete nuclei, however, do not constitute a chemical union but a mechanical mixture. The numerical reduction of the chromosomes is a consequence and a condition of sexuality. It is probably not a mere halving of the bulk of the chromatin, but a selection and a distribution between daughter cells of structural entities—the primordia of characters which are handed from one generation to another. The Mendelian principle shows, if it shows anything worth while, that these units act independently. The nucleus, therefore, directs and controls cellular development. The outer manifestations known as variation are probably due to the inter action of nucleus and cytoplasm.

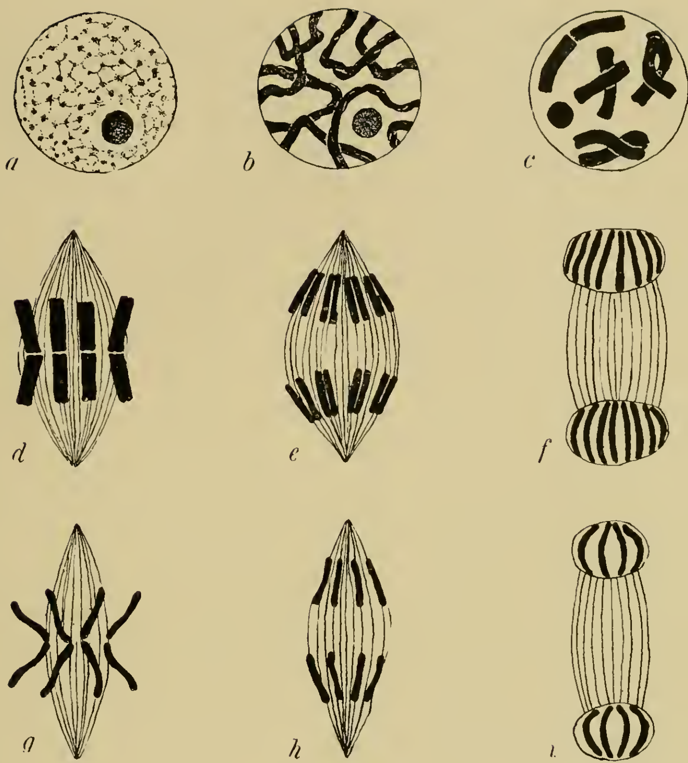

Fig. 2.

Fig. 2. Diagrams illustrating the behavior of the chromatin during the two maturation divisions in a spore mother cell. *a—f.* first or heterotypic mitosis. *a.* resting nucleus, same as in fig. 1. *b.* longitudinally split chromatin spirem developed from *a*; the halves of the spirem are twisted upon each other. *c*, spirem has segmented into eight chromosomes which have approximated in pairs to form the four bivalent chromosomes. These eight chromosomes were united end to end in the spirem of *b*, just as in the ordinary somatic mitosis. *d.* spindle with the four bivalent chromosomes arranged in the equatorial plate. *e.* anaphase, the four chromosomes retreating towards the poles of the spindle. Each of these retreating chromosomes is now more clearly seen to be composed of two halves which were formed by the longitudinal splittiug in *b*. *f.* daughter nuclei in which the spirems will be formed by the union end to end of the daughter segments. This is the division in which the number of chromosomes is reduced to one-half, because whole chromosomes pass to each daughter nucleus. If these whole chromosomes are different in hereditary characters, the division is qualitative or differential. *g—i.* second or homotypic mitosis. *g.* spindle showing the four chromosomes arranged in the equatorial plate; the free ends of the daughter segments of each chromosome diverge from each other. *h.* the segments passing to the poles of the spindle. *i.* the grand-daughter nuclei resulting from the second mitosis. This is an equasional division, because it consists of the separation of half chromosomes, or daughter segments, formed by the longitudinal fission of whole chromosomes.

In speaking of sex, let us bear in mind that among both animals and plants there may be three kinds of individuals: Dioecious species, in which the individuals are unisexual, either male or female; monoecious, with bisexual or hermaphrodite individuals; and parthenogenetic, in which individuals produce eggs that develop without fecundation. We may now take up the question, whether the sex of the individual is determined by factors of the environment, or is it predetermined in the chromatin of the sex cells, i. e., in either sperm or egg or both? Of the environmental factors, that which is supposed to play the most important role is nutrition, and in the case of plants, it is probably the only one that need be considered, for other important factors, such as light and heat, are only influential in so far as they affect nutrition. But we should also understand that we have two sorts or two categories of environmental conditions. In case the fecundated egg develops wholly apart from the parental body, and as a completely independent individual, its supply of nourishment is from the external world; but in those cases in which the incubation of the fertilized egg and the early development of the embyro take place within the parental body, the food supply will depend upon the condition of the parent. While the conditions of these two categories seem very different, yet it will be found that the final results are essentially the same.

For the sake of simplicity, a few instances from the animal kingdom will be mentioned. Experiments were carried on by Riley and others to determine whether the starving of caterpillars of butterfles might influence the number of males and females; for under normal conditions of nutrition the caterpillars produce both males and females, and because it is not possible, says Riley, to make caterpillars take more food than they do naturally. The results of the experiments showed that an excess or diminution of food does not alter the proportion of the sexes. Upon this point Morgan (Exp. Zool., p. 377) makes the following statement: "The futility of many of these experiments has now become apparent, since it has been shown that the sex of the caterpillar is already determined when it leaves the egg. Under these circumstances it is not probable that feeding could produce a change in the sex. It is much more probable that starvation or overfeeding could only affect the proportion of males and females by bringing about a greater mortality of the individuals of one sex." Numerous studies have been made upon the silk worm by Kellog and Bell, and by Cuneot upon flies and moths, to determine the influence of food conditions upon the sex of the individual and upon that of the egg and sperm, with the conclusion that the sex is not determined by external conditions. While the preponderance of evidence along this line seems to argue strongly against any influence upon sex-determination by food conditions, yet there is one case, that of Hydatena senta and the daphnid, Simocephalus, investigated by Nussbaum and others, in which it seemed probable that food might have some determining influence. Maupas, on the other hand, regarded temperature and not food as the influential factor. In this connection, the studies of von Malsen (Archiv. f. mikr. Anat., 69: 63-97. 1906.) upon a small worm, Dinophilus apatris, and of Issakowitsch (Idem) upon daphnids, are of especial interest. Von Malsen found that a higher temperature (26° C) was favorable to the development of males, while a lower temperature gave an increased ratio of females. He does not attribute the change in the sex ratio to the temperature directly, but indirectly as affecting the nutrition of the animal. The amount of food at the disposal of the animal was the same, but at the higher temperature, the sexual activity of the animal, i. e., the rapidity with which a large number of eggs was produced, was abnormally accelerated, so that the bodily nutrition was insufficient for the proper nourishment of the eggs. Consequently, at a higher temperature a larger number of eggs are produced, and among them is a proportionately large number of smaller or male eggs. At a lower temperature, on the contrary, reproductive activity was slower, and among the smaller number of eggs developed, a larger ratio of well nourished female eggs was the result. There was more time for the development of these eggs, and consequently more food placed at their disposal. To estimate the value of these statements it is necessary to examine the data upon which the conclusions are based. The number of eggs considered and the sexual ratio in the warm and cool cultures are shown in the following tables:

NORMAL.

No. of Eggs.	*Male.*	*Female.*	*Ratio of Male : Female.*
1140	327	813	1 : 2.4

Number of eggs at each laying, 5.6.

COOL.

No. of Eggs.	*Male.*	*Female.*	*Ratio of Male : Female.*
3948	973	2975	1 : 3.5

Number of eggs at each laying, 4.2.

WARM.

No. of Eggs.	Male.	Female.	Ratio of Male : Female.
1393	507	886	1 : 1.7

Number of eggs at each laying, 3.6.

At the higher temperatures it commonly happened that the eggs were developed so rapidly that the body of the animal was entirely filled from one end to the other, the head appearing as a smallpoint, the intestine so compressed as to be scarcely visible. In this condition the animal is unable to move and soon perishes. At the higher temperature, therefore, a larger number of eggs are produced so rapidly that the body can not properly nourish them. It seems to me that von Malsen's conclusions should be accepted with much reserve, because of certain probable sources of error. In the first place he seemed to have based his estimate of males and females upon the size of the eggs alone, the large ones representing females, the smaller eggs males. From the very marked variation in the size of the female eggs, as given from his own measurements, it would seem that size alone would not be a strictly accurate method of determining the sexes. In the second place it does not seem improbable that, at higher temperatures, and with a more rapid generative activity, fewer smaller eggs would fall as prey to the larger eggs; for in these animals the larger female eggs are frequently nourished at the expense of the smaller. If the nutritive activity of these large eggs is increased proportionately to the sexual activity by higher temperature, then the larger eggs should consume the smaller ones in like ratio; but von Malsen does not seem to have shown this to be true. It may be said that at lower temperatures the larger female eggs have relatively more time in which to consume the smaller, hence fewer small, or male, eggs are laid. The question then arises, does von Malsen's experiments prove that higher temperature leads to the production of more female than male eggs from the generative tissue? or merely that, at a higher temperature, of the relatively larger number of eggs produced, a proportionately smaller number of male eggs is consumed in the nutrition of the female eggs.

The researches of Issakowitsch upon a daphnid bring us face to face with a different class of data. This author reared the descendants of parthenogenetic females through several generations (six as a maximum), and found that the production of females is paralleled with high temperature (24° C.), and that the males with lower temperature, the direct opposite to that which happened in the worm Dinophilus. Issakowitsch shows that temperature acted merely as influencing nutrition, for when the animals were starved by being reared in distilled water, males and resting eggs were developed From his experiments it would seem that, so far as parthenogenetic eggs were concerned, nutrition may act as a sex-determining factor. Both von Malsen and Issakowitsch look upon nutrition as a sex-determining factor from the influence it is supposed to produce upon the plasmic relation in the nucleus, as set forth by Richard Hertwig.

The more recent researches of Punnett upon Hydatena seem to throw new light upon the subject in that they point out probable errors in the studies of Maupas and Nussbaum (R. C. Punnett: Sex-determination in Hydatena, with some remarks on parthenogenesis. Proc. Royal Soc., Series B., 78: 223, 1906). In Hydatena three kinds of females may be recognized by the kinds of eggs they lay: (a) females which produce females parthenogenetically (thelytokous females); (b) females which produce males parthenogenetically (arrenotokous females); and (c) the layers of fertilized eggs. Of the first class of females, Punnett recognized from pedigree cultures three different types. A. Females giving rise to a high percentage of male producing individuals (arrenotokous females). B. Females giving rise to a low percentage of male producing individuals. C. Purely female producing individuals (pure thelytokous females).

In experiments designed to test the effect of temperature and nutrition, it was found that in the purely female producing individuals (class C), no male producing forms appeared, the strain remaining pure, and that in the class B, the ratio of males was not raised as a result of starving. Consequently it is difficult to see that either temperature or nutrition has any influence in determining male producing forms. Punnett suggests "that the females, producing females parthenogenetically (thelytokous), are really hermaphrodite, though the male gametes may not exhibit the orthodox form of spermatozoa. Such a view would account for the observed absence of polar bodies in the female eggs, for it must be supposed that the process of reduction and fertilization takes place before the accumulation of yolky material." It may be added also that if no polar bodies are formed, there is no reduction in the number of chromosomes, and we may have, as has been clearly shown in certain plants, not a case of parthenogenesis but one of apogamy. Whitney (Whitney, David Day. Determination of sex in Hydatena senta. Jrnl. Exp. Zool., 5: 1-26, 1907), in a still more recent study of Hydatena senta, finds that neither temperature nor nutrition has anything to do with the determination of sex. He asserts also that the three strains of Punnett can be found in one strain and each is capable of producing the other types according as the data is scanty or extensive.

Even if we admit that the results obtained with certain animals furnish some evidence in favor of the view that nutrition may be instrumental in determining sex, yet the vast majority of facts obtained from numerous studies made upon lower and higher plants point unmistakably to the opposite conclusion. I shall mention a few instances. Botanists have long recognized the difficulty of obtaining for class use the zygospores, or the sexually formed reproductive bodies, in the common bread mould Rhizopus nigricans, and this was supposed to be due to the lack of knowledge of the external conditions necessary to call forth sexual reproduction. Blakeslee has recently shown that this common mould is dioecious, and that if male strains are cultivated along with female strains, sexual reproduction will take place irrespective of external conditions; whereas if the different strains are grown separately, no zygospores will result, no matter what the food conditions may be Again, the well-known liverwort, Marchantia, produces male and female sexual organs upon separate thalli, or individuals. These individuals are propagated by bodies called gemmae, and it is reported that Noll has cultivated individuals from the gemmae under all sorts of growth conditions without being able to change the sex of any of the thalli. The thalli arise primarily from spores that are apparently all alike, and that come from the same capsule, yet some of these spores must be strictly male and others female. The well-known studies of Prantl upon fern prothallia are frequently quoted as supporting the doctrine that food conditions determine sex. Prantl found that under poor conditions of nourishment the prothallia produced only male organs, and if removed to conditions affording good nourishment, female organs were developed. In this as in many similar cases, there was no change of sex since monoecious organisms were operated with, that is organisms capable of producing both male and female gametes. Lack of nourishment merely inhibited the development of the tissue upon which the female organs are borne, and consequently only male organs were developed. These prothallia arise from spores that contain the characters of both sexes, and external conditions merely stimulate the development of one or the other of the sexes, or both.

The writer has recently begun the study upon a fern, whose prothallia have been reported as strictly dioecious, and that if the spores are well nourished female prothallia will predominate, while with poor nourishment the vast majority of spores will give rise to male gametophytes. An examination of cultures grown under favorable conditions for laboratory use, in which the spores were sown thickly, showed that certain spores produced strictly male plants, others female, and still others bisexual prothallia. A small number of spores were isolated and grown under similar and very favorable conditions, with similar results. The pure males were almost equal in number to those bearing the female organs, while the bisexual plants were few, being about four per cent. of the whole number. The foregoing results seem to lend encouragement to the view that environmental conditions may have much less to do with the development of male and female prothallia than had hitherto been supposed. The very brief study showed clearly that in the fern in question there is a great mortality among the spores, which, as can be readily seen vary greatly in size. Among the first things to establish in this and similar cases is whether mortality is greatest among the smaller or larger spores, and whether the prothallia springing from the small spores tend to remain small and produce only antheridia, while the larger female plants arise only from the larger spores, an so on. I have no notion what sort of results a careful and extended study will bring forth.

Of all efforts to ascertain the influence of the environment upon the determination of sex, doubtless the studies carried on upon dioecious plants by Strasburger and many others are the most noteworthy. Especially interesting and instructive in this connection is a representative of the pink family, the Red Campion, Lychnis dioica, which is attacked by a smut, Ustilago violaceae, whose spores are produced in the anthers instead of pollen. This red campion is dioecious, certain individuals bearing only staminate and others pistillate flowers. The structure of the staminate and pistillate flowers are shown in the following figure.

If a plant, bearing staminate flowers, be infected by the smut, the anthers when mature will be filled with smut spores instead of pollen. Apart from the color of the anthers the form of the staminate flower is unchanged by the presence of the parasite. On the other hand, if a plant, bearing pistillate flowers, is befallen by the smut, the blossoms on the branches affected by the smut, will develop normally appearing stamens, whose anthers are filled with smut spores instead of pollen, while the pistil remains in a rudimentary condition. The only apparent difference between a pistillate flower thus affected by the parasite and the normal staminate blossom is an elongation of the axis between calyx and corolla (Fig. 3b′). At first sight it might appear that the presence of the parasite was sufficient to change the sex of the plant, for the fungus, when present in the pistillate plant, leads regularly to the development of stamens and the suppression of the pistil. However, in this case the capacity to develop stamens must be assumed to be present in the pistillate plant, and the parasite is able to induce the conditions necessary to their formation and the suppression of the pistil, and thus provide for the development of its own spores. Extensive and elaborate experiments by Strasburger upon uninfected plants with the view of duplicating the effects produced by the parasite, led to no definite results.

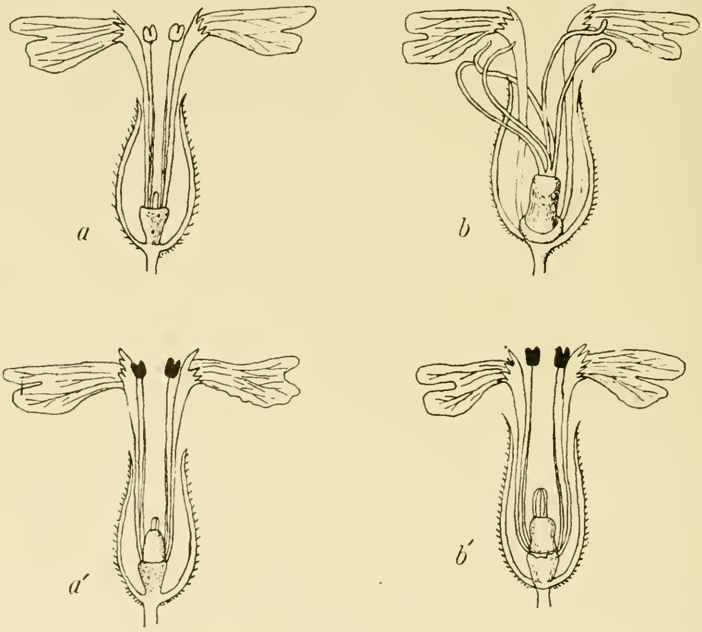

Fig. 3.

Fig. 3. Staminate and pistillate flowers of Lychnis dioica L., halved longitudinally. *a*. normal staminate flower. *b*. normal pistillate flower. *a′* staminate flower affected by the smut, Ustilago violaceae; the anthers contain smut spores instead of pollen. *b′* pistillate flower similarly affected: the pistil has remained rudimentary while anthers have been developed, which, however, bear only smut spores. The presence of the parasite has induced the development of anthers, the members of the flower bearing male spores instead of the parts bearing the female spores,—After Strasburger.

Laurent (1903) has maintained that an excess of nitrogen or lime favors the development of males in spinach, hemp, etc., while potash and phosphoric acid favor the development of females, but his results are not very convincing. Temperature, light and moisture conditions, relative age and vigor of parents, relative maturity of pollen, early and late planting, pruning, etc., have all been carefully and elaborately tested without achieving satisfactory or convincing results. The case of the anther smut cited in the foregoing seems to furnish the best evidence among plants that the sex of the spores to be developed can be changed by environmental conditions, yet it must be admitted that the preponderance of evidence is against the view that environmental conditions, either direct or indirect, can determine sex. On the other hand, there are many who believe that sex is predetermined in the germ cells, and that we are confronted with a problem which is purely hereditary. According to this view certain parts of the hereditary substance or chromatin contain male characters, or repesent maleness only and certain other parts female characters, or femaleness, that is, there are male determinants and female determinants in the chromatin. To illustrate this statement, let us recall the case of the common liverwort, Marchantia. Of the spores produced by any individual sporophyte, some will give rise only to male thalli and others to female thalli irrespective of environmental conditions. Now, the spores producing only male plants must contain only male determinants, or male determining parts of the chromatin must dominate over the female determinating parts in those spores and vice versa. If the determination ot sex be regarded as a problem of heredity, and if we believe that hereditary phenomena have a physical basis, some such theory as the foregoing certainly affords a rational basis for further investigation.