

CHAPTER VIII

THE TETRAPLOIDS, OR FOURFOLD TYPE

THE chromosomes have been counted in more than a thousand species of animals and probably in as many or more species of plants. In two or three species only one pair of chromosomes is present. At the other extreme there are species with over one hundred chromosomes. No matter how many or how few the chromosomes, the number is found to be constant for each species.

It is true that irregularities in the distribution of the chromosomes occasionally take place. Most of these irregularities are, as a rule, automatically straightened out in one or another way. It is also true that, in one or two cases, a slightly variable number of chromosomes may occur, as in *Metapodius* where one or more small, extra chromosomes, sometimes the Y-chromosomes, sometimes another chromosome called the M-chromosome, may or may not be present (Fig. 62). As Wilson has shown, these chromosomes may, perhaps, be looked upon as indifferent bodies that have lost their importance, since there are no corresponding variations in the characters of the individuals.

It is known, furthermore, that chromosomes may join together, decreasing the number by one or more, but the totality of the genes is still preserved, and this also holds for cases where a chromosome may break, increasing for a time at least the number by one.¹ Finally, there are

¹ The occasional breaking apart of chromosomes in *Oenothera* has been described by Hance. In the moth *Phragmatobia*, and in other moths also, Seiler has described several cases where certain chromosomes that are united

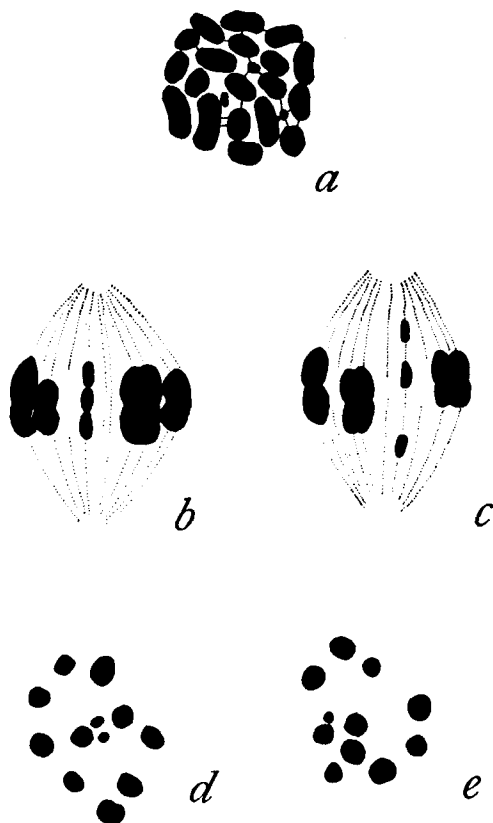


FIG. 62.

Chromosomes of *Metapodius*. *a*, spermatogonial group with three small *m*-chromosomes; *b* and *c*, side view of spermatocytes, conjugation of three *m*'s, two pass to one pole, one to the other, as seen in *d* and *e* (anaphase plates of *c*). (After Wilson.)

species where the female has one chromosome more than the male, and there are other species where the in the eggs and sperms, are separate in the embryonic cells. In the bee each chromosome is supposed to break into two parts in all of the somatic cells. In some of the tissue cells of flies and other animals the chromosomes may divide without the cell dividing and in this way become doubled or quadrupled.

reverse may be true. All these situations have been extensively studied, and are familiar to every student of the cell. The occurrence of such cases does not invalidate the general statement, *that the number of the chromosomes is constant and characteristic of each species.*²

In recent years an ever increasing number of cases has been reported in which individuals have suddenly appeared that have double the number of chromosomes characteristic of the species. These are the fourfold types, or tetraploids. Other multiple types have also been found, some arising spontaneously, others derived from the tetraploids. We speak of these collectively as polyploids. Of these polyploids, the fourfold group is in many ways the most interesting.

In animals there are only three cases of tetraploidy that are certainly known. The parasitic threadworm of the horse, *Ascaris*, occurs in two types, one with two and one with four chromosomes, respectively. These two varieties are like each other, even as to the size of their cells. The chromosomes of *Ascaris* are regarded as compound and as formed by the union of a number of smaller chromosomes sometimes called chromomeres. In the cells of the embryo that will become body-cells, each chromosome breaks up into its constituent elements (Fig. 63, a, b, d). These are constant in number or approximately so,

² Della Valle and Hovasse have in recent years denied that the number of chromosomes is constant in different tissue cells. In so far as the conclusions are based on an examination of the somatic cells of Amphibia that have a large number of chromosomes difficult to identify with accuracy, their results do not suffice to overthrow the overwhelming number of observations on other forms (and even on some Amphibia) where the number of the chromosomes can be accurately determined.

It is also known that in certain tissues the number of the chromosomes may be doubled or quadrupled, either by failure of cells to divide when the chromosomes divide, or else by the chromosomes breaking up into a constant number of parts. These are special cases that do not affect the general situation.

and there are in all about twice as many elements in bivalens as in univalens. This supports the view that there are twice as many chromosomes in one type as

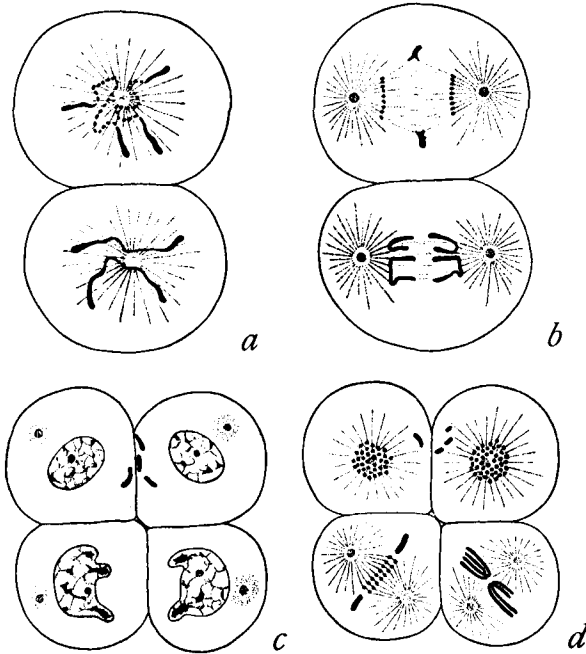


FIG. 63.

First and second cleavages of the egg of *Ascaris univalens* with two chromosomes. In *a* and *b* the fragmentation of the two chromosomes in one of the cells is shown. In *d*, three cells show fragmented chromosomes, while in the fourth cell the chromosomes are intact. The latter gives rise to the germ-cells. (After Boveri.)

in the other, rather than that bivalens has arisen through the halving of the univalens chromosomes.

One form of the brine shrimp, *Artemia salina*, is, according to Artom, a tetraploid. There are two races, one with 42 chromosomes, the other with 84 chromosomes (Fig. 64). The latter propagates by parthenogenesis.

Under these circumstances it is not difficult to imagine that the tetraploid originated in a variety that was already parthenogenetic, for, should an egg-cell double the number of its chromosomes by the retention of one of its polar bodies, or become double through the chromosomes failing to separate after the first division of a nucleus, the double condition might continue to perpetuate itself.

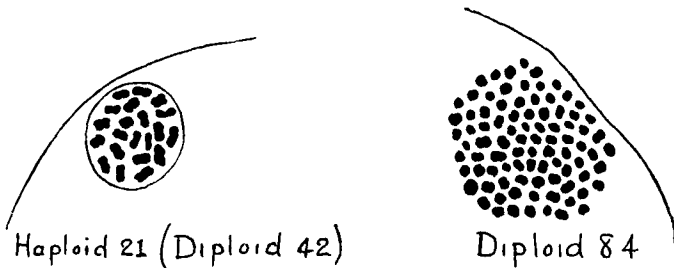


FIG. 64.

The chromosomes, in reduced number, of the diploid and tetraploid races of *Artemia salina*. (After Artom.)

One of the first tetraploids in plants was discovered by de Vries, and named *Oenothera gigas* (Fig. 42). It was not known, at first, that this giant was a fourfold chromosome type, but de Vries saw that it was stouter than plants of the parent species (Lamarck's evening primrose) and different in many other minor characteristic details. Its chromosome number was later made out.

Lamarck's evening primrose (*Oenothera Lamarckiana*) has 14 chromosomes (haploid 7). The giant form *O. gigas* has 28 chromosomes (haploid 14). The two chromosome groups are drawn in Fig. 65.

Gates has made measurements of the cells of different tissues. The epidermal cells of the anthers of *gigas*

have almost four times the volume of the normal type; those of the stigma three times the volume; those of the petals twice the volume and the pollen mother cells are about one and a half times larger. The nuclei of the latter have, in *gigas*, twice the volume of the parent type. The cells in the two types also differ sometimes markedly in their superficial dimensions. Most species of evening primroses have 3-lobed discoidal pollen grains, some of those of *gigas* are 4-lobed.

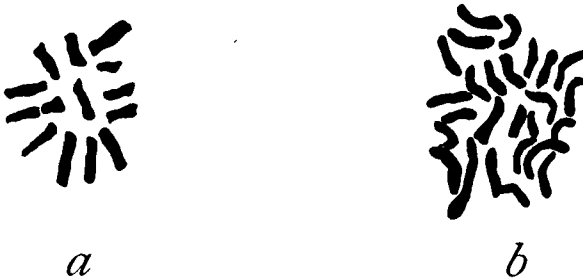


FIG. 65.

a, The fourteen diploid chromosomes of *Oenothera Lamarckiana*;
b, the twenty-eight diploid chromosomes of *O. gigas*.

The maturation of the pollen mother cells has been studied by Gates, Davis, Cleland, and Boedijn. Gates reports that in *O. Lamarckiana* there are, as a rule, 14 pairs of bivalent chromosomes (gemini) in the giant. At the first maturation division, half of each bivalent goes to each daughter cell. At the second division each chromosome splits lengthwise and gives 14 chromosomes to each pollen grain. A similar process presumably occurs in the ripening of the ovules. Davis describes the chromosomes of *O. Lamarckiana* that emerge from the synaptic tangle as stuck together somewhat irregularly and not strictly in side to side union. Later they move toward one

or the other pole bringing about reduction. Cleland has recently described an end-to-end union of the chromosomes of another diploid species, *Oenothera franciscana*, as they enter the maturation spindle (Fig. 66). Some of

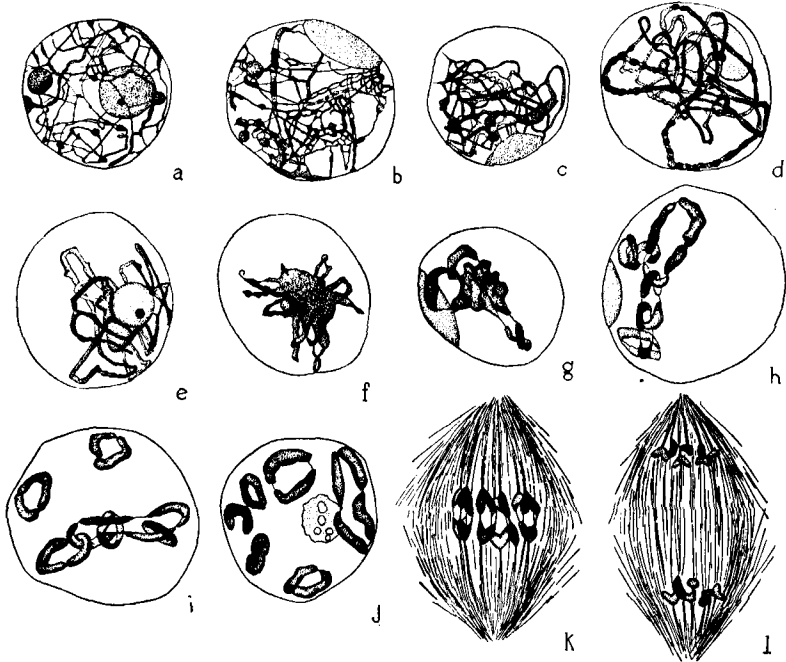


FIG. 66.

The maturation of the pollen cells in *Oenothera franciscana*.
(After Cleland.)

the earlier figures of Davis had also to some extent indicated an end-to-end union.

In other monoecious flowering plants tetraploids have also been found in recent years. It is obvious why these occurrences should be more frequent in monoecious species than in species with separate sexes; for, in the

former, eggs and pollen are produced on the same plant. Hence if a plant has started as a tetraploid, it will produce both egg-cells and pollen-cells with a diploid number of chromosomes. Self-fertilization will give tetraploids again. On the other hand, in animals or plants with separate sexes the eggs of one individual must be fertilized by sperm from another individual. Now, if a tetraploid female should arise, her ripe eggs, with the diploid number of chromosomes, will ordinarily be fertilized by the haploid sperm from a normal male, with the result that a threefold type, or triploid is formed. From a triploid the chance of recovering a tetraploid again is very small.

The tetraploids that have arisen in pedigreed cultures furnish more accurate information as to their origin than do tetraploids found accidentally. There are, in fact, other records where tetraploids have arisen under controlled conditions. In *Primula sinensis*, Gregory has found two giant types, one of which appeared in a cross between two diploid plants. Since the parent plants contained known genetic factors, Gregory was enabled to study the inheritance of the characters in the fourfold type. His results left him undecided as to whether they indicated that a given member of each of the four like chromosomes unites with a specific mate or equally with any member of its group. Muller's analysis of the same data indicates the latter as the more probable conclusion.

Winkler has obtained a giant nightshade (*Solanum nigrum*) and a giant tomato (*Solanum lycopersicum*) through the intermediate process of grafting, which has in itself, so far as known, no direct relation to the production of the double forms.

The tetraploid nightshade was obtained in the following way. A piece of a young tomato plant was grafted into a young nightshade plant from which the axial buds were then removed. A cross cut was made, ten days later,

at the graft level (Fig. 67). Adventitious buds grew up from the callus tissue of the exposed surface. One of these plants was a chimaera, *i.e.*, a plant part of whose tissue was nightshade and part tomato. It was removed and propagated. Some of the axial buds of the new plant

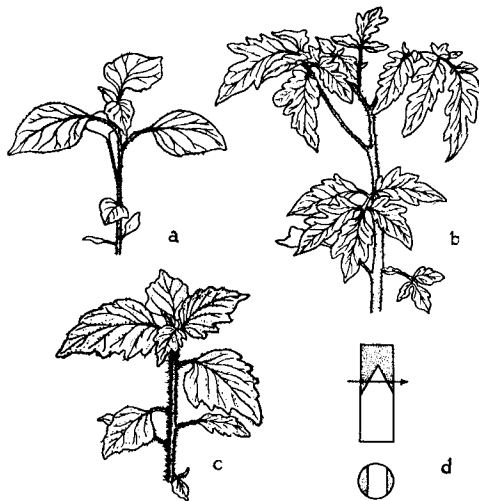


FIG. 67.

a, Seedling of *Solanum nigrum*. b, Seedling of *Solanum lycopersicum*. c, *Solanum tubingense*—graft-hybrid. d, method of grafting. (After Winkler.)

had a tomato epidermis and a nightshade core. These branches were then isolated and planted. The plantlets differed from other chimaeras known to be diploid, which created a suspicion that the new type might have a tetraploid core, which was confirmed by examination. The tops of these chimaeras were cut off, and the axial buds of the basal half removed. From the adventitious buds of the

callus, young plants were obtained that were tetraploid throughout. One of these gigas nightshade plants is shown in Fig. 68, to the right, and a normal (diploid) or parent type, to the left; a flower of gigas is shown above to the right in Fig. 69 and the parent type to the left. A seedling gigas is shown and a seedling of the parent type above left, Fig. 69.



FIG. 68.

Normal diploid parent plant of solanum to the left, and tetraploid to the right. (After Winkler.)

The differences in the cells of some of the tissues are shown in Fig. 69. The palisade cells of the leaf of the gigas type and the corresponding cells of the parent type are shown below to the left; the guard cells of the gigas stomata and those of the parent type are shown below to the right; the hairs of the gigas form and those of the parent are shown at the bottom to the right; the pith cells of the giant are correspondingly larger than those of the normal plant. The pollen grains of the giant are repre-

sented in the middle to the right and those of the parent type to the left.

A tetraploid tomato plant, also, was obtained as follows. A piece of a young tomato plant was grafted on to a stock of nightshade in the usual way (Fig. 67). After union

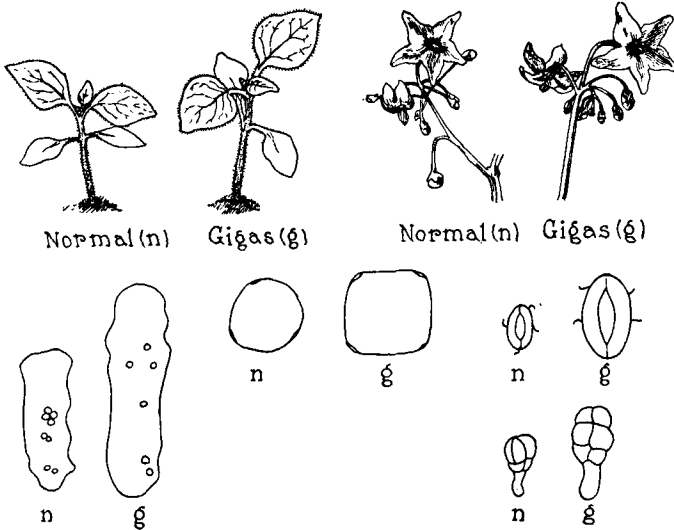


FIG. 69.

Diploid and tetraploid seedlings and flowers of the nightshade are shown above, and tissue cells below. Above to left, seedlings; above to right, flowers; below to left, palisade cells; in middle, pollen grains; to right stomata, above, and hairs below. (After Winkler.)

had been perfected, a cut was made across the union of the two plants and the axial buds removed from the stock. From the cut surface, young buds developed in the callus tissue. These were removed and planted. One of these had an epidermis of nightshade cells and a core of tomato cells. It was found on further examination that the epidermal cells were diploid and the cells of the core were

tetraploid. In order to obtain, from this composite plant, a tetraploid in all of its parts, the stem of a young plant was cut across and the axial buds below the cut were removed. New adventitious buds appeared on the cut surface which were, for the most part, made up of the

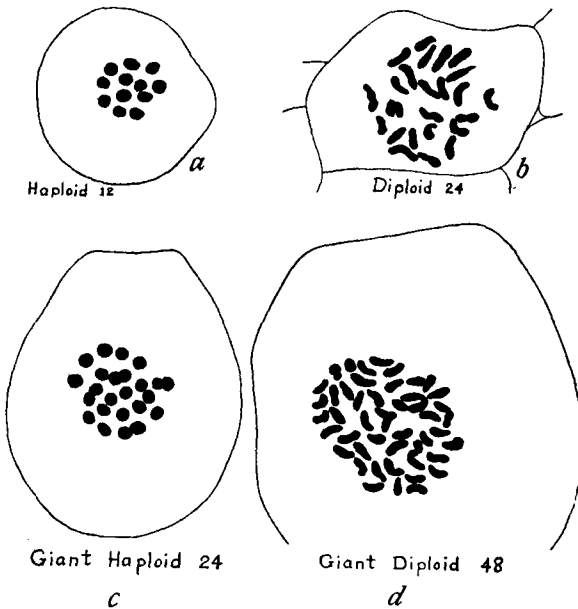


FIG. 70.

a, Haploid; *b*, diploid cell and chromosomes of nightshade; *c*, haploid, and *d*, diploid cell and chromosomes of tetraploid nightshade. (After Winkler.)

tomato tissues both within and without. The giant tomato plant differs from the parent plant in the same way as does the giant nightshade from its parent.

The diploid nightshade has 24 chromosomes, its haploid number is 12; the tetraploid has 48 chromosomes, and its haploid number is 24 chromosomes. The diploid tomato has 72 chromosomes (haploid 36). The tetraploid

tomato has 144 chromosomes (haploid 72 chromosomes). These chromosomes are shown in Figs. 70 and 71.

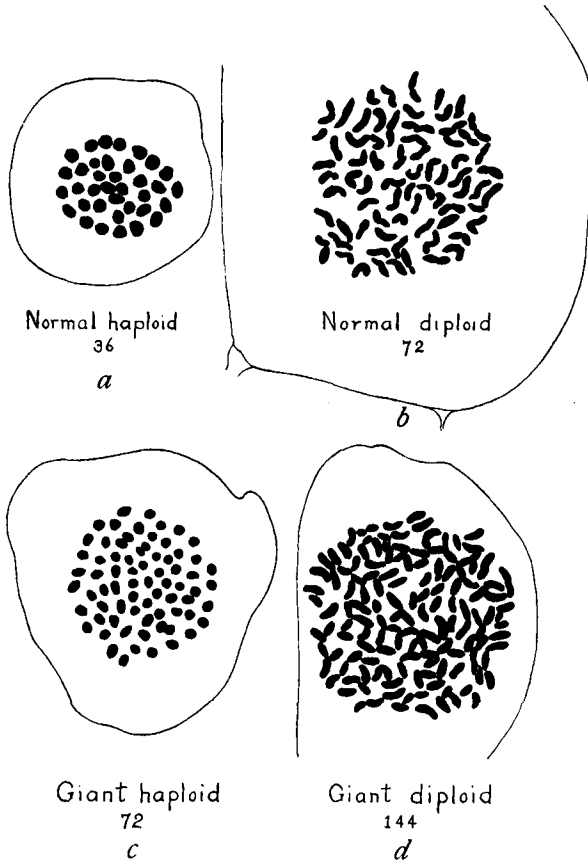


FIG. 71.

a, Haploid; *b*, diploid cell and chromosomes of tomato; *c*, haploid, and *d*, diploid cell and chromosomes of tetraploid tomato. (After Winkler.)

As has been said, there is no obvious relation in these cases, as far as known, between grafting and the formation of tetraploid cells in the callus. How these cells arise is uncertain. It is possible that two cells of the callus fuse

together, as Winkler at one time thought probable, but it seems more likely that the tetraploids arise by the suppression of the cytoplasmic division of a dividing cell, which would thereby double the number of its chromosomes. Such a tetraploid cell might form the whole or only the core, or any other part of a young plant.

A tetraploid of the common Jimson weed (*Datura stramonium*) (Fig. 72 below) was found by Blakeslee.

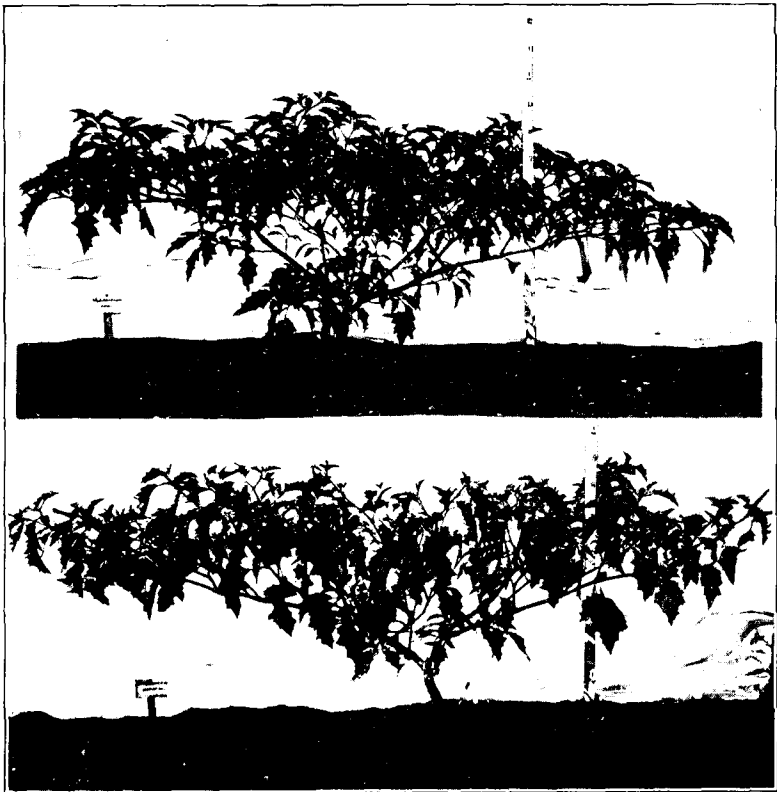


FIG. 72.

Diploid plant of *Datura stramonium*, above, and tetraploid, below.
(After Blakeslee.)

Belling, and Farnham. In appearance it is described as differing in several respects from the diploid type. The differences in the capsule, flower, and stamens in the diploid (second column) and tetraploid (fourth column) are shown in Fig. 73.

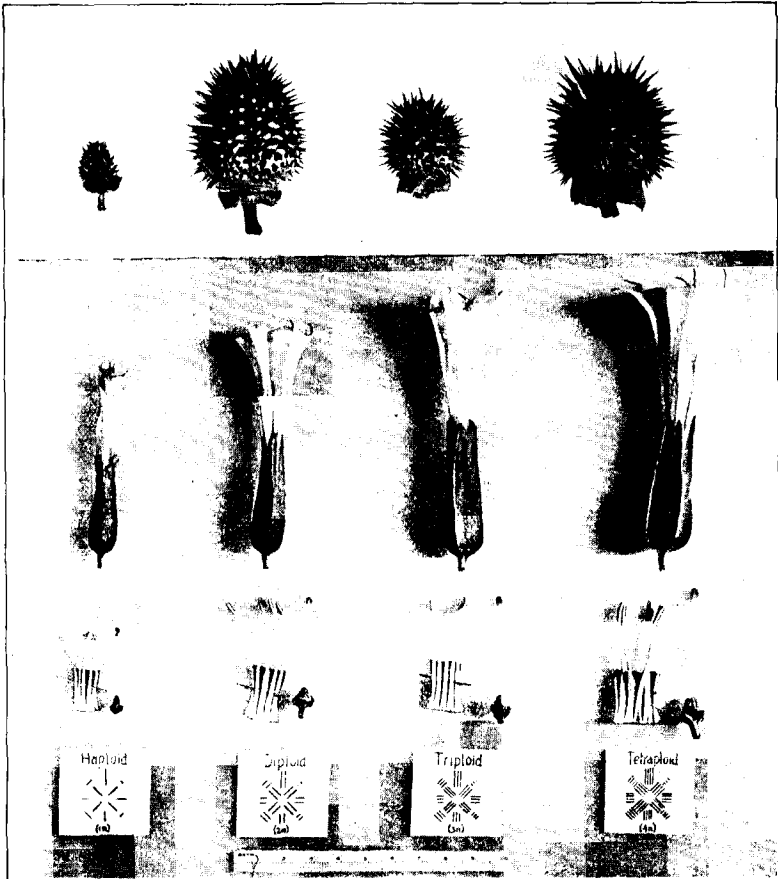


FIG. 73.

Capsules, flowers, and stamens of haploid, diploid, triploid, and tetraploid *D. stramonium*. (After Blakeslee, in *Journal of Heredity*.)

The diploid plant has 12 pairs of chromosomes (24 chromosomes) which according to Belling and Blakeslee can be arranged in six sizes (Fig. 74), namely, large (L and l), medium (M and m), and small (S and s), or $2(L+4l+3M+2m+S+s)$. The formula for the haploid

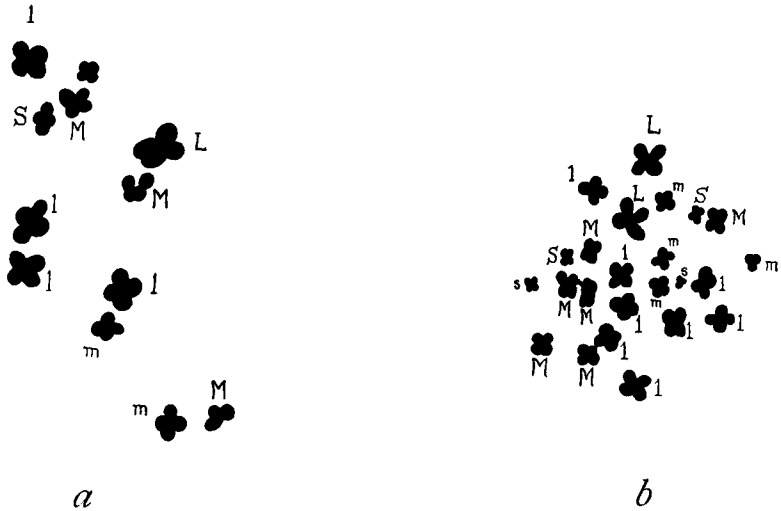


FIG. 74.

a, Second metaphase chromosome group of diploid *Datura stramonium* with 12 chromosomes (each constricted); and *b*, corresponding group of tetraploid with 24 chromosomes. (After Belling and Blakeslee.)

group is $L+4l+3M+2m+S+s$. These chromosomes, when about to enter the first maturation division (prophase), form pairs of rings or else are united by one end (Fig. 75, second column). One conjugant of each pair then moves to one pole and its mate to the opposite pole. Preparatory to the second maturation division, each chromosome constricts, producing the appearance shown in Fig. 74b. One constricted half passes to one pole of the spindle,

the other half to the other pole. Each daughter cell gets 12 chromosomes.

The tetraploid has 24 pairs or 48 chromosomes. Prior to their entrance into the first maturation spindle they come together in fours (Fig. 76 and Fig. 75). The differ-

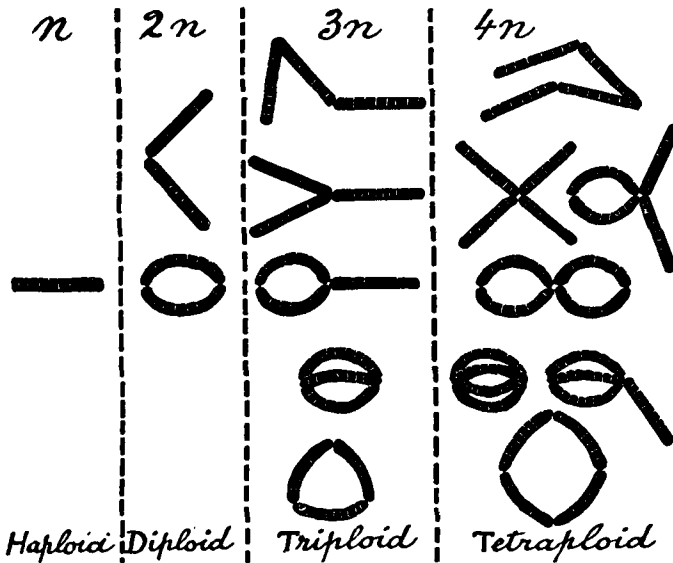


FIG. 75.

Methods of conjugation of the chromosomes in diploid, triploid, and tetraploid types of *Datura stramonium*. (After Belling and Blakeslee.)

ent ways in which these chromosomes are combined in these quadrivalent groups is shown in these figures. They enter the first maturation spindle in approximately this condition. At the first maturation division two members of each quadrivalent pass to one pole and two to the opposite pole (Fig. 75). Each pollen grain has 24 chromosomes. Occasionally, however, three chromosomes may pass to one pole and one to the other.

The 24 chromosomes of the tetraploid at the second maturation division are shown in Fig. 74. They resemble those of the diploid at the same stage. Half of each passes to one pole, half to the opposite pole. Belling records that in 68 per cent the distribution is regular, *i.e.*, 24 to each pole (24+24). In 30 per cent of cases the dis-

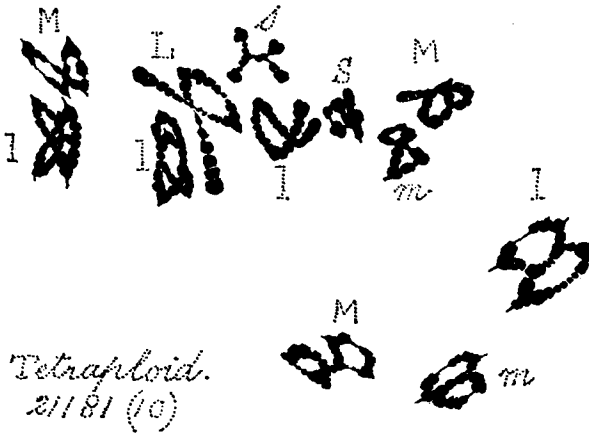


FIG. 76.

Conjugation of the chromosomes of the tetraploid of *Datura stramonium*. Four like chromosomes unite to make up each group. (After Belling and Blakeslee.)

tribution gives 23 at one pole and 25 at the other (23+25). In 2 per cent there were 22 at one pole and 26 at the other. In one case the distribution was 21-27. The result shows that irregularities of distribution are not uncommon in the tetraploid *Datura*. A further test of this was made by self-fertilizing a tetraploid. The progeny was grown to maturity and the chromosomes in their germ-cells counted. The number of chromosomes in 55 of the plants was 48; in five plants it was 49; in one plant it was 47; in another it was 48 (?). If the distribution in the egg-cells is like that in the pollen cells, it follows that the

germ-cells with 24 chromosomes are those most likely to survive and function. Some of these plants with more than 48 chromosomes might give new types with still greater irregularities of distribution of the chromosomes, owing to the additional extra chromosomes.

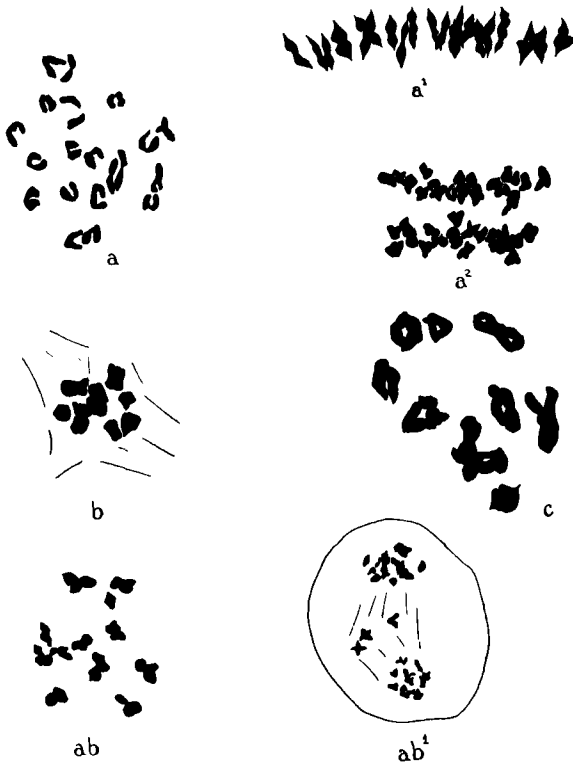


FIG. 77.

a, *Euchlaena perennis*, first maturation division, prophase; with 19 bivalents and two single chromosomes, a', Metaphase of last. a'', Anaphase of same. b, *Zea mays*, first maturation prophase with ten bivalents. c, *Euchlaena mexicana*, first maturation division, prophase, with ten chromosomes. ab, Hybrid (F_1) between *E. perennis* and *Zea mays*, prophase of first maturation division with 3 trivalent, 8 bivalent, and 5 single chromosomes. ab' Same as last, late anaphase of first maturation division. (After Longley.)

A tetraploid *Narcissus* has been reported by de Mol. The diploid species has 14 chromosomes (7 pairs) while two cultivated varieties were found to have 28 chromosomes. De Mol points out that until 1885 the small diploid varieties were chiefly cultivated. Then the larger triploid types appeared and finally about 1899 the first tetraploid was obtained.

The perennial teosinte of Mexico has twice as many chromosomes as the annual teosinte, according to Longley. The perennial Fig. 77a, has 40 chromosomes ($n=20$) and the annual 20 chromosomes ($n=10$) Fig. 77c. Longley crossed both of these with corn (maize), that has 20 chromosomes ($n=10$) Fig. 77b. The hybrid between the annual teosinte and corn has 20 chromosomes. At the maturation stages of the pollen mother cells there are 10 bivalents, and these divide and pass to the poles without any lagging chromosomes. This means that the 10 chromosomes that have come from the teosinte conjugate with the 10 that have come from the corn. When the perennial teosinte is crossed to corn the hybrid has 30 chromosomes. At the ripening of the pollen mother cells of the hybrid the chromosomes are found to be united, some in threes, others in twos; the rest have no partners (Fig. 77ab). This leads to irregularities in the division that follows (Fig. 77ab¹).

In hermaphroditic or monoecious plants, where the question of sex determination is not involved with differential sex-chromosomes, the tetraploid may be said to be both balanced and stable. By balanced is meant that the numerical relations of the genes is the same as that in the diploid or normal type. By stable is meant that the mechanism of maturation is such that the type, once established, perpetuates itself.³

Tetraploids in mosses were produced as early as 1907

³ Blakeslee used the terms differently.

by Élie and Émile Marchal by artificial means. Each moss plant has two generations, a haploid protonema stage (gametophyte) that produces eggs and sperm-cells and a diploid stage (sporophyte) that produces asexually

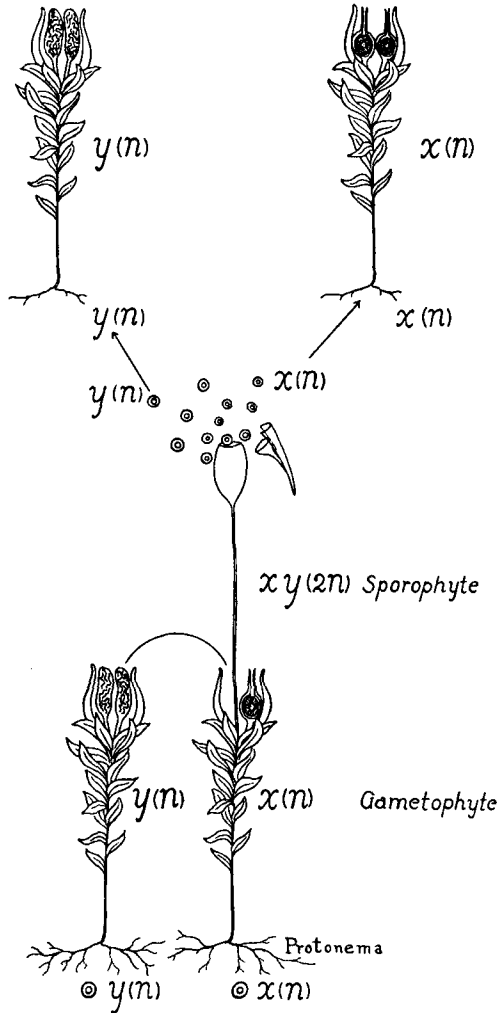


FIG. 78.

Normal life cycle of dioecious moss.

the spores (Fig. 78). Pieces of the sporophyte if kept under moist conditions give rise to threads whose cells are diploid. These become a true protonema that gives rise in time to diploid eggs and diploid sperm-cells. By the union of these germ-cells tetraploid sporophyte plants are formed (Fig. 79). Here the normal haploids have been duplicated by a diploid protonema and moss plant, and the diploid sporophyte has been duplicated by a tetraploid sporophyte.

The Marchals have made comparative measurements of the size of the cells of the normal plants and of those of the tetraploids. In three species the volume of the normal perianth cells to that of the doubles was found to be as 1 to 2.3; 1 to 1.8; and 1 to 2. The volumes of the cells of the normal antheridia in the two types were as 1 to 1.8 and those of the nuclei were about as 1 to 2. The egg-cells were as 1 to 1.9. Measurements of the antheridial organs (that carry the sperm-cells) and of the archegonial organs (that carry the egg) showed in all cases that the double types are longer and broader than are the normal types. It is evident that the increase in size of the double types is due to larger cells and these in turn have larger nuclei, which, other evidence has shown, have in the double types twice as many chromosomes as in the normal type. This was, of course, to be expected from their origin by regeneration from the normal sporophyte.

In the sporophyte generation the mother cells of the $2n$ spores were to those of the $4n$ spores about as 1 to 2.

The two maturation divisions in mosses, *i.e.*, the divisions following conjugation of the chromosomes, take place in the sporophyte at the time when the spores are formed—four from each spore mother cell. If, in mosses, the chromosomes carry the genes, the doubling of the chromosomes (tetraploid) in double types is expected to

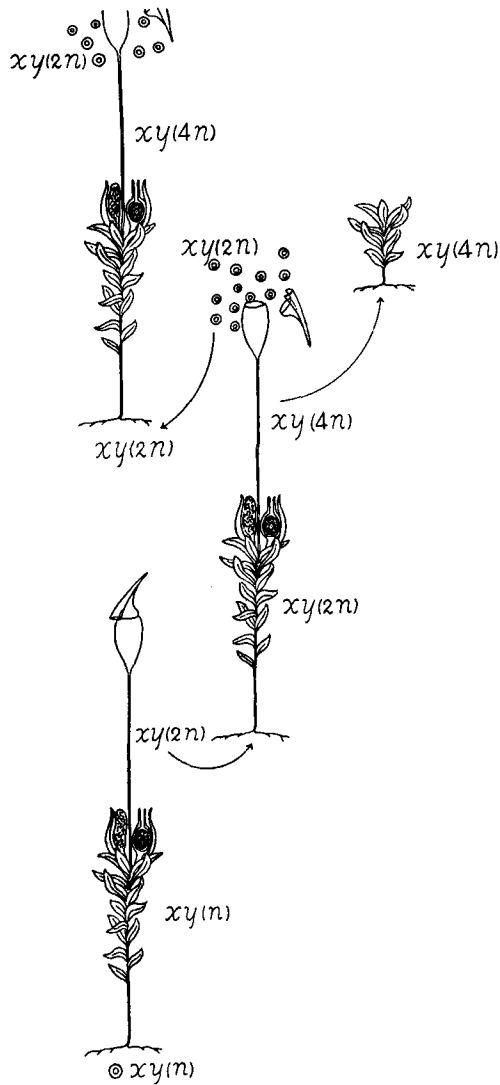


FIG. 79.

Formation of a diploid protonema ($2n$) by regeneration from a $2n$ sporophyte of a normal, monoecious moss. By self-fertilization a $2n$ gametophyte gives rise to a tetraploid or $4n$ sporophyte. By regeneration from the latter a tetraploid gametophyte is produced.

give ratios different from those in the normal plants. As yet little has been done in this direction, although Wettstein has found clear evidence of genetic inheritance in a few species crosses of mosses, and Allen, in the related group of liverworts, has genetic evidence for two characters of the gametophyte.

In those mosses with separate sexes and in certain liverworts it has been shown by the Marchals, by Allen, by Schmidt, and by Wettstein that the sex-determining elements are sorted out at the time of spore formation. An account of these observations and experiments will be given in the chapter on sex.

There are many important questions for embryology rather than for genetics relating to the size of the cells of tetraploids. In general it may be said that the cells are larger, and frequently twice as large, but there is a good deal of variation in the different tissues in these respects.

The size of the whole plant as well as some of the other peculiarities of the tetraploid are due apparently to the increase in size of its cells. If this is correct, it means that these characteristics are developmental rather than genetic. The way in which tetraploids arise has to some extent been already considered. The methods that have been suggested, as to how the increase in the amount of cytoplasm in the cells of the tetraploids takes place, call for further examination.

If two cells in the germ-track should fuse, and their nuclei then or later unite, a tetraploid cell might result. If the double cell continued to maintain a double volume in the growth period, an egg of twice the normal size would be expected to result. The number of cells of the larger embryo would also be expected to be the same as that characteristic of the normal embryo.

There is, however, another possibility, namely, that the

double germ-cell might not be able to increase to double size in the germ-track of its diploid mother. The egg might not then be any larger than the normal egg, but have twice as many chromosomes. The embryo developing from this egg might not be able to get enough nourishment to increase the size of its cells until the post-embryonic or larval stages were reached, when food is obtainable from the outside. Whether at this late period the presence of a double set of chromosomes in each cell would bring about an enlargement of the cytoplasm of each cell is uncertain. In the next generation, however, the eggs would develop from the beginning with a four-fold set of chromosomes in a tetraploid body, and under these circumstances it is conceivable that the egg might grow to double size before dividing.

It is even less to be expected, perhaps, that an immediate increase in amount of the cytoplasm could take place if the doubling of the chromosomes occurred in a mature egg after it is fertilized. The embryos of animals pass through a rather definite number of cell-divisions before organ formation begins. If an embryo should start as an egg of normal size but with double the number of chromosomes, and if, in consequence of the double number present, cleavages should cease sooner than in the normal egg when organ formation sets in, such a tetraploid embryo would then have cells twice the size of the *normal embryo but only half as many cells.*

In the flowering plants where ample space and food supply is present in the embryo sac, the development of an egg with a larger amount of cytoplasm may have a more favorable chance to take place.

Tetraploidy as a Means of Increasing the Number of Genes in a Species.

One of the most interesting considerations connected with tetraploids from an evolutionary standpoint is the opportunity they may seem to furnish for increasing the number of new genes. If new and stable types arise through doubling the number of the chromosomes, and if, after doubling, the four like chromosomes should become different in the course of time, so that two become more like each other, and the other two also become more like each other, the tetraploid would then resemble genetically a diploid, except in so far as many of the genes remained unchanged. Many like genes would then be present in four chromosomes of each set, and the expectation for the F_2 , when an individual is heterozygous for only one pair of genes, would be a Mendelian ratio of 15 to 1 instead of 3 to 1. Such ratios have in fact been found (wheat, shepherd's purse) but whether tetraploidy accounts for the result or whether doubling has occurred in some other way remains to be determined.

On the whole, it seems that until we know something more as to the way in which new genes arise—if they do now arise—it is rather hazardous to take advantage of tetraploidy as a general explanation to account for a change in number of the genes. It is true that in monoecious plants new types may arise in this way, yet it is improbable that, in animals with separate sexes, tetraploidy could become established (except in parthenogenetic species), because, as has been pointed out above, the tetraploid is lost by crossing to an ordinary or diploid individual and not easily recovered afterwards.