VARIATIONS IN DATURA DUE TO CHANGES IN CHROMOSOME NUMBER

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Two forms with which we have recently carried on breeding experiments, the garden flower *Portulaca* and the jimson weed (*Datura Stramonium*), are strikingly different in the types of variations which they show. The *Portulaca* is procurable in a wide range of color varieties, and is apparently subject to relatively frequent mutations, both seminal and somatic, with sectorial and periclinal chimeras a common phenomenon. Sufficient breeding tests have been made to indicate that the varieties of *Portulaca* are due in large measure at least to gene mutations. In comparison with *Portulaca,* the jimson weed is relatively stable so far as gene mutations are concerned. Despite the large amount of breeding work with this species, both before and since the rediscovery of Mendel's law, only the two allelomorphic pairs of characters, purple *vs.* white flowers, and spiny *vs.* smooth capsules, have been identified aside from the pair, tall *vs.* short stature recently determined by the writer and Avery (3).

It is true that certain of our pure lines of *Datura* differ slightly from others when grown in comparable pedigrees, but the fact remains that so far as sharply contrasting Mendelian characters are concerned, the jimson weed is highly stable, while the *Portulaca* is highly mutable. Our knowledge of changes in chromosome number in other forms is not sufficient to indicate if there is any significance for the present discussion in the difference just mentioned between *Portulaca* and *Datura.*

Our interest in *Datura* began about 1910 or 1911, when the jimsons were used as demonstration material for students in genetics. In 1915 we found our first mutant which we called the Globe from the shape of its capsules.

The capsules of normal plants are ovate and the edges of the leaves somewhat toothed. Globe plants, on the contrary, have depressed capsules and broader leaves with a more entire margin (cf. 3, figs. 7 and 9). Figure 1 shows

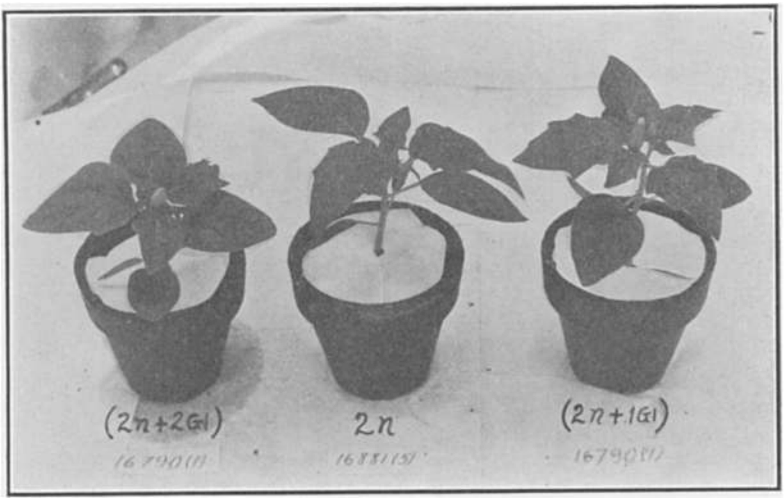

FIG. 1. Young plants in 3-inch pots. The normal $2n$ plant is in the middle, the $(2n+1)$ Globe on the right, and the $(2n+2)$ Globe on the left.

young plants beginning to flower. In the center is a normal and on the right a Globe. The leaves of the latter are broader and more closely massed together. In the plant on the left, the Globe characters are more strongly developed. This plant represents an extreme type of the Globe mutant, and has been called the Round-leaf Globe. It is of considerable genetic interest and will be discussed later. It was at first thought that the Globe might be a tetraploid type like the Gigas *Œnothera* but a preliminary cytological investigation showed that such was not the case.

A peculiarity in the inheritance of the Globe (1, table 3) was found to be that the Globe complex is transmitted to only about one fourth of its offspring when a Globe parent is selfed; that about the same proportion of one fourth Globes only appears in the offspring when the Globe parent is crossed with pollen from a normal plant; and that the mutant character is transmitted to only a slight extent or not at all through the pollen—to less than 2 per cent. in a large series of crosses.

The next mutant found was Cocklebur (3, fig. 11) named from the resemblance of its fruits to those of the cocklebur weed. The plant is weak and lopping and the leaves narrow and twisted.

The Poinsettia mutant (3, fig. 14) was named from a fancied resemblance of its long clustered leaves to the hothouse plant of that name. The Poinsettia is of especial interest, since this mutant was found to give curious ratios when heterozygous for color factors.

As our eyes became better trained, other mutants were added to the list, largely through the keen discrimination of Mr. Avery and Mr. Farnham, until we now have 12 main mutants with some varieties, all of which transmit their mutant characters essentially in the same way in which the Globe complex was found to be transmitted.

In addition we had a mutant which, unlike the 12 types just mentioned, was found to breed true, and since it is practically impossible to obtain crosses between it and the normal form from which it arose, it was called "New Species" (3, fig. 15). The capsules are somewhat spherical and the leaves broad, although in a race of the same type later discovered the leaves are not greatly different from the normals. Heterozygous plants of the "N. S." sometimes gave curious ratios in their offspring.

Such was the situation up to the spring of 1920, when we were fortunate in securing the cooperation of Mr. Belling in a study of the nuclear condition of our mutants. On the basis of his work we are able to make the classification of types shown in Fig. 2. In the individual figures—which of course are highly diagrammatic—the chromosomal constitution of somatic cells is represented. We have not attempted to represent the size differences determined by Mr. Belling and pictured in our paper in the morning session.[1] A word of explanation of terms is desirable. The terms diploid, triploid and tetraploid are already current to indicate a balanced condition in which each chromosomal set (we can not say chromosomal

[1] To be published shortly in the AMERICAN NATURALIST.

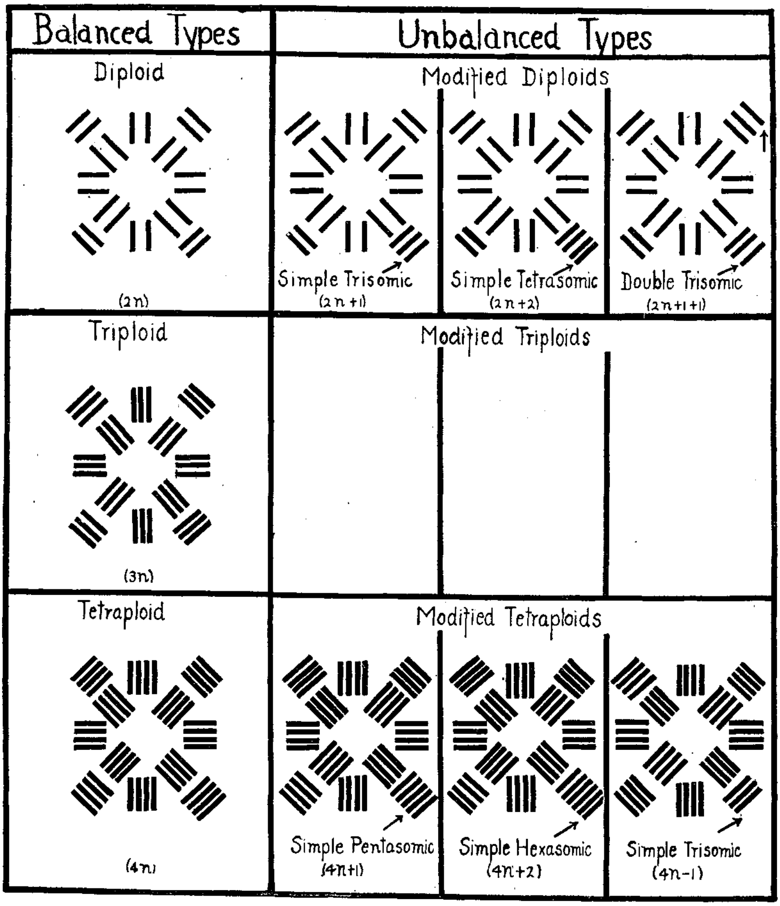

FIG. 2. Diagrams illustrating the chromosomal types already found in *Datura*.

pairs when there are more than 2 in a set) has respectively 2, 3, or 4 chromosomes. I have suggested (2) the terms disome, to indicate a set of 2 chromosomes, trisome a set of 3, and tetrasome a set of 4, etc., with the adjectives disomic, trisomic, tetrasomic, etc. Such terms may be found useful, but it seems impossible to devise a simple terminology that will adequately describe even the chromosomal irregularities at present known in *Drosophila* and *Datura*. Accordingly, after considerable discussion with Dr. Bridges, we have agreed upon a set of formulæ which is illustrated in the diagram and which we shall use in our present papers.

Of the balanced forms there are even-balanced or stable, and odd-balanced or unstable types. In the even-balanced diploid, which is the normal jimson weed, the two chromosomes in each set go to opposite poles by the ordinary process of disomic reduction, and the plants breed true for chromosome number. Partly for the same reason, the even-balanced tetraploid, which is our "New Species," breeds essentially true. The triploid, on the other hand, is odd-balanced and therefore unstable, since in the trisomic disjunction in each set two of the three chromosomes go to the one pole and one to the other, the process taking place at random. Through the operation of chance, therefore, gametes of different chromosomal number will be formed, and simple and double mutants as well as diploids will occur in the offspring. The relation may be seen from the pollen of the three balanced types under the same magnification (Fig. 3), where the photograph at the left (*a*) shows a field of pollen from a diploid; that at the right, (*c*) with larger grains, pollen from a tetraploid; while that above (*b*) shows pollen from a triploid. Pollen from a triploid is not only characterized by a large proportion of empty grains, but also by a great diversity in the size of the grains brought about by the differences in the number of chromosomes which they contain.

The upper left-hand figure of the unbalanced types (Fig. 2) has one extra chromosome in the lower right-hand set, indicated by the arrow, giving 1 trisome, and 11 disomes in this nucleus, and its formula may be written $(2n+1)$. Such a simple mutant is the Globe—simple because only one set is affected. If another set has the extra chromosome—say the set on the right—instead of the one with the arrow, this extra chromosome would cause the plant to assume the characters of, say, the Cocklebur mutant. It is obvious that since there are 12 sets in *Datura* and each set may have an extra chromosome, there are 12 mutants with the formula $(2n+1)$ theoretically possible. Through the process of disjunction in these 12 mutants, half of the gametes should contain the extra chromosome, and half should not. Differential mortality, affecting adversely zygotes with the extra chromosome, prevents the expected equality of ($2n$) and ($2n+1$) individuals in the offspring from test crosses with diploids.

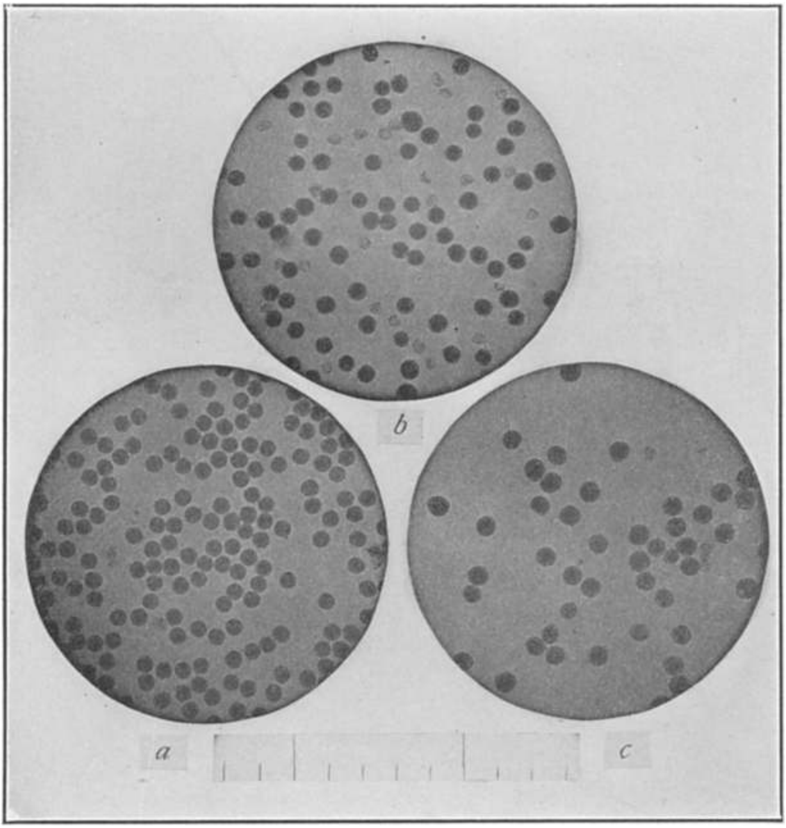

FIG. 3. Photomicrographs of pollen grains: (*a*) from a diploid *Datura;* (*b*) from a triploid; (*c*) from a tetraploid. The magnification is indicated by the scale, each division of which equals 0.10 mm.

The 12 mutants under discussion may best be represented in a single figure by their capsules. In Figure 4 we have capsules of the 12 simple trisomic mutants viewed from the ovate side, each one of which represents the addition of a single extra chromosome presumably in a different set. There is the Globe with depressed capsules and stocky spines; the large long-spined Poinsettia; the narrow short-spined Cocklebur; the slender-spined Ilex;

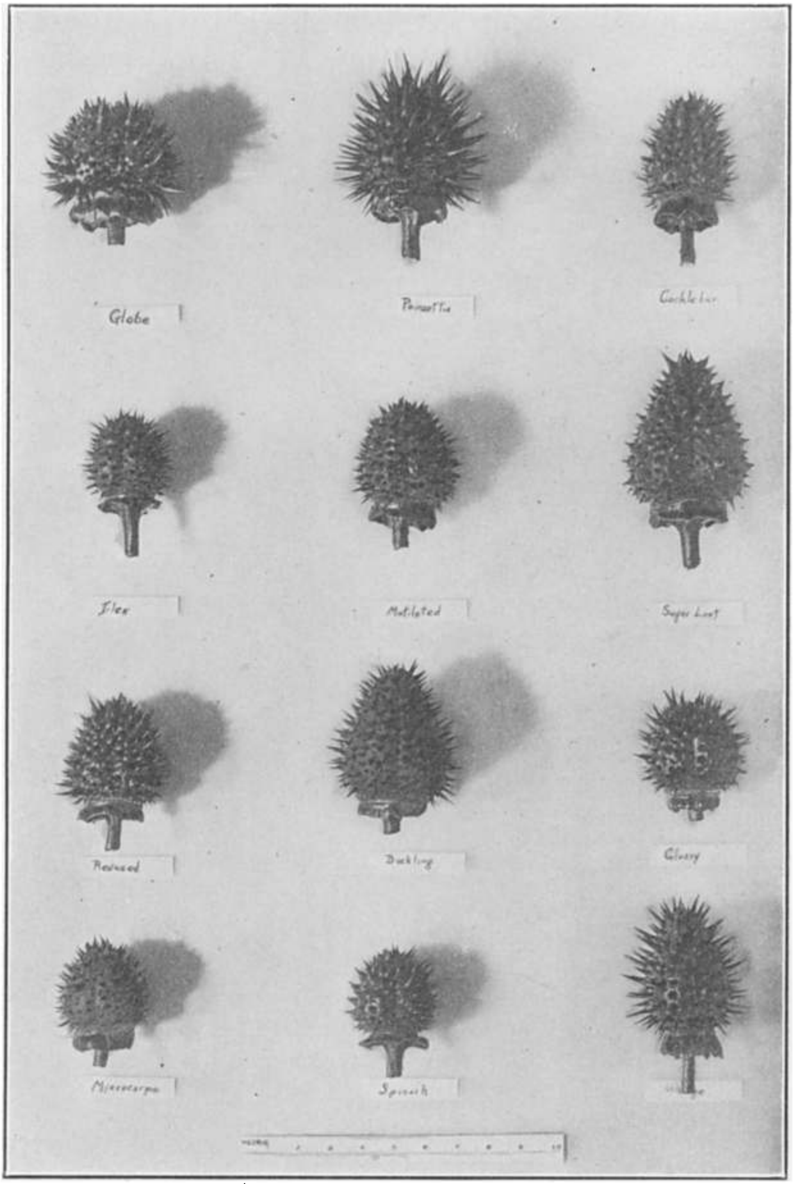

FIG. 4. Photographs of capsules of 12 mutants of *Datura* viewed from the ovate side.

the Mutilated, usually mutilated with a diseased blotch; the short-spined Sugarloaf; the shiny capsule of Glossy, etc., with lastly the narrow, long-spined Wedge. I have provisionally called these mutants the 12 apostles. Certain of the 12 have varieties which may be called acolytes, and perhaps some of these in the figure may be reduced from the rank of apostles to that of acolytes when other forms are discovered.

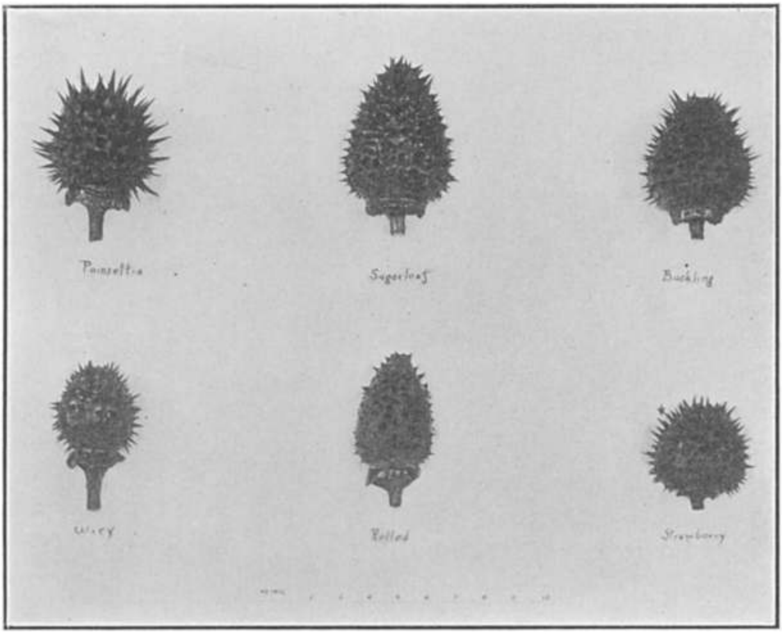

FIG. 5. Capsules representing 3 pairs of mutants. Those in the lower row are believed to represent varieties or "acolytes" of the respective types represented above.

In Figure 5, the mutants from which the capsules in the lower row were taken have been provisionally classed as acolytes of their respective apostles represented above. The evidence is best in regard to the mutants Wiry and Poinsettia which form the pair at the left in the figure. They both contain a single extra chromosome of approximately the same size, and in both cases this extra chromosome is shown, by peculiar color ratios in their offspring, to be in the set which carries the factors for purple and white flower color. The fact that though perfectly distinct they are yet similar in appearance, and the fact that one has not infrequently given rise to the other in our cultures, is a line of argument applicable not alone to the pair Wiry and Poinsettia. It also leads us to consider Rolled an acolyte of Sugarloaf, and Strawberry an acolyte of Buckling. The possibility of acolytes being caused by modifying Mendelian factors is being investigated.

I have said that the Poinsettia mutant gave curious color ratios in its offspring (4 and 2, table 2). The evidence seems conclusive that Poinsettia has its extra chromosome in the set which carries the factors for purple and white flower color. A heterozygous Poinsettia may have one dose or two doses of the dominant purple flower color. The offspring of Poinsettia (like those of the Globe), it will be remembered, are part normals and part mutants. If a Poinsettia parent is duplex for purple, its normal offspring show 8 purples to 1 white, while its Poinsettia offspring are all purples. If the Poinsettia parent is simplex for purple the ratio for the normal offspring is 5 purples to 4 whites, and for Poinsettia offspring is 7 purples to 2 whites. The back crosses are also distinctive. By similar reasoning we believe the Cocklebur mutant has its extra chromosome in the set which carries genes for presence or absence of spines on the capsules.

The evidence is especially good for Poinsettia, since the color classes can be recognized in the seedpan. Using a Poinsettia which arose in a purple line from Washington, D. C., we crossed it with a white line of similar appearance also from Washington, and, without going outside of these two lines, have synthesized Poinsettias of all the possible combinations of color factors and have made nearly all the possible combinations of crosses between them. The results with the Washington lines are in accord with what would be expected from a random assortment of 3 chromosomes in the set containing the purple-white color factors. In a certain group of Poinsettias simplex for purple in which the 2 chromosomes bearing the white factor might have been brought in, so far as we knew, either from the white Washington stock, or from a distinct white line from Erfurt, Germany, the color ratios in the offspring of some parents were according to calculation, but from other parents the whites were approximately 6 times as frequent as would be expected. Later experiments seem to indicate: that we get the definite excess in white offspring from simplex parents when both the "white" chromosomes come from the German line; that we get the Poinsettia ratios typical of random assortment when the two white chromosomes come from the Washington whites; and that we get both of the two types of ratios from different individual F_2 parents when we make up an F_1 Poinsettia containing both a Washington white, and a German white chromosome. It is apparent that the peculiarity must be attributed to the German chromosomes. The question is receiving further experimental investigation but our provisional hypothesis to account for the difference in the ratios is that for some reason in trisomic disjunction the German white chromosomes go to opposite poles rather than to the same pole 6 times as frequently as the laws of random assortment would dictate.

Let us return to our diagrams in Fig. 2. Of the modified diploids we may have 2 extra chromosomes in a single set forming a simple mutant of the formula $(2n+2)$. An example is the round-leaf Globe (fig. 1) already mentioned.

If two different sets are affected each with a single extra chromosome we have a double mutant with the formula $(2n+1+1)$. Of the 66 different double trisomic mutants theoretically possible, we have a considerable number now under cultivation. As an example, the double mutant Globe-Reduced is shown in Fig. 6. At the top is a capsule of a normal diploid with its chromosomal diagram. At the left is a capsule of the Globe, and at the right a capsule of Reduced. Their diagrams indicate that the two mutants have different sets affected. The plant represented by the capsules below, from the appearance of its leaves as well as from that of its fruit, is undoubtedly a double mutant with the two sets affected as indicated in the diagram below. If the Globe-Reduced behaves like other double mutants we have bred, its offspring should contain normal diploids, both the Globe and the Reduced mutants, as well as the double mutant, Globe-Reduced, roughly in the proportion of 6: 2: 2: 1.

Triploids (fig. 2) have been discussed in this morning's session. Our prediction at last year's meeting has been

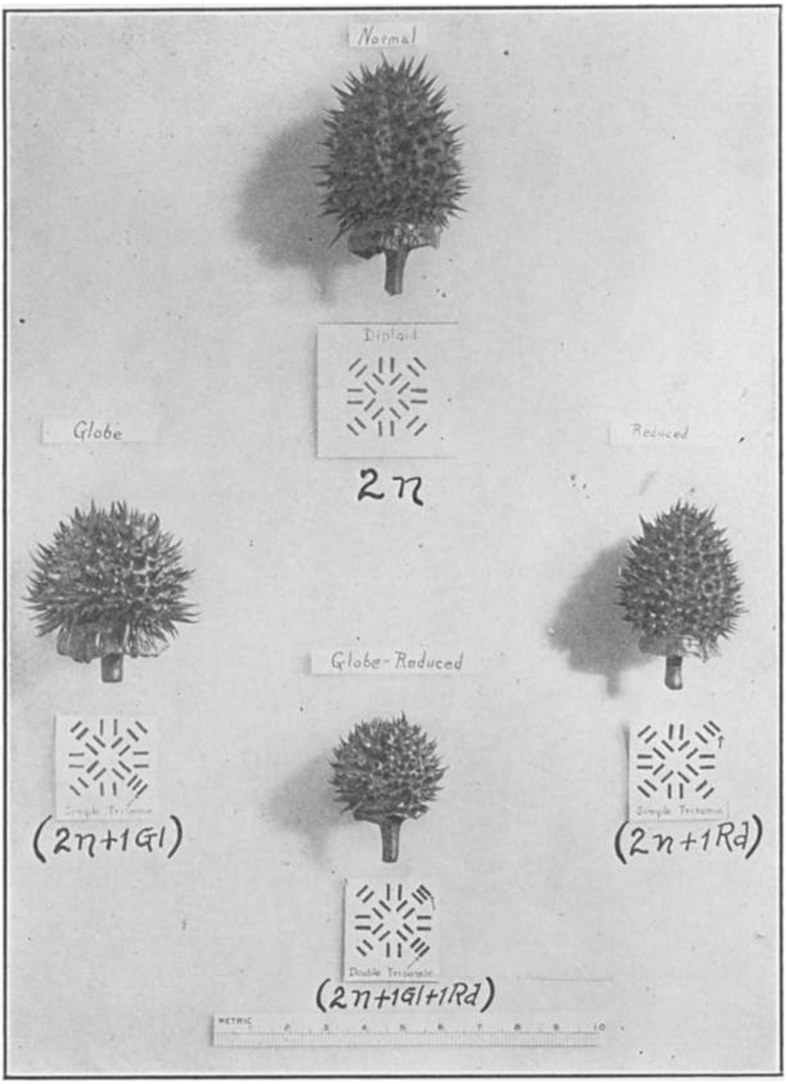

FIG. 6. Capsule of normal diploid ($2n$) above; capsule of Reduced ($2n+1$ *Rd*) at right; capsule of Globe ($2n+1$ *Gl*) at left; and capsule of double mutant Globe-Reduced ($2n+1$ *Gl* $+1$ *Rd*) below. Below each capsule is given its chromosomal diagram.

fulfilled and we have obtained, in the offspring of a triploid, practically the full range of $(2n+1)$ mutants as well as double mutants of the formula $(2n+1+1)$. No modified triploids have as yet been identified, but even if we found them we could not expect to be able to propagate them by seed.

Heterozygous tetraploid plants also show curious ratios, according to whether there are 1, 2, or 3 doses of the dominant factor. Duplex plants give a 35: 1 ratio when selfed and the different types in the offspring segregate in a characteristic fashion.

In the tetraploids we may have a single extra chromosome in one set making a simple $(4n+1)$ mutant, or 2 chromosomes in a set making a simple $(4n+2)$ mutant. We have two cases of a tetraploid with a deficiency in one set, producing a $(4n-1)$ mutant.

Up to the present time, except for Gregory's work on tetraploid *Primulas* (5) which was correctly interpreted by Muller (6), Mendelian research has dealt almost exclusively with disomic inheritance. Our work with the jimsons and the recent investigations of Bridges on triploid *Drosophilas* offer an opportunity for the rather novel study of trisomic, tetrasomic and pentasomic inheritance. We do not believe, however, that the jimson weed is peculiar among plants in giving rise to chromosomal mutants.

The unbalancing effect of the extra chromosomes can best be illustrated by extra chromosomes in the Globe set. The $(2n+2)$ Globe has two extra chromosomes in the Globe set and hence should show a greater divergence from normal than the Globe with only one extra chromosome. Such is the case. The simple $(2n+1)$ Globe (like other mutants of this type) is less vigorous in growth than normals. The $(2n+2)$ Globe is still less vigorous than the more common $(2n+1)$ Globe. From fig. 1 it will be seen, further, that the Globe characters in the $(2n+2)$ Globe on the left, such as broadness of leaves, fatness of bud, and density of foliage, are much further developed than in the $(2n+1)$ Globe at the right, which has only one extra chromosome.

Photographs of capsules (Fig. 7) will further illustrate the idea of unbalance. Unfortunately the $(2n+2)$ Globe just mentioned fruits poorly and none of its capsules were available when the fruits of the other types were photographed. Later a photograph of a capsule was made to the same scale, and inserted in the proper place in the series. It will be evident that the Globe characters of relative stockiness of spines and depression of capsules are more marked in the $(2n+2)$ Globe where there are 2 extra chromosomes in the Globe set than in the $(2n+1)$ Globe on the left where there is only one extra chromosome in this particular set. Likewise in the modified tetraploids the (plus 2) Globe on the right is more Globe-like than the (plus 1) Globe beside it.

The degree of unbalance of chromosomes in the nuclei may be given a quantitative expression. Thus in the $(2n+1)$ Globe, the extra chromosome produces an excess of one over the balanced $2n$ condition. The nucleus is overbalanced by the active factors in a single Globe chromosome. This unbalance may be said to be 1 over $2n$. In a similar way the $(2n+2)$ Globe with 2 extra chromosomes has an unbalance of 2 over $2n$. Having in mind these quantitative differences one would expect the $(4n+1)$ Globe with an unbalance of 1 over $4n$ to show a less marked expression of the Globe characters than the $(2n+1)$ Globe with an unbalance of 1 over $2n$. They are, in fact, less readily recognized in recording our pedigrees. The relation of unbalance enabled us to predict the possibility of finding $(4n+2)$ Globes with an unbalance of 2 over $4n$ which one would expect to be as distinct in appearance as $(2n+1)$ Globes with an equivalent unbalance of 1 over $2n$. The prediction has been fulfilled and we are led to expect the appearance of Globes with 3, and Globes with 4 extra chromosomes in the Globe set, if tetraploid plants can endure the extreme unbalance of 4 over $4n$, the equivalent of 2 over $2n$ obtained in the $(2n+2)$ Globe.

It must be emphasized that our quantitative expressions of unbalance hold strictly only for the chromosomal numbers in reference to a single set, and not necessarily for the somatic characters conditioned by them, although the nuclear unbalance seems to be reflected in the somatic

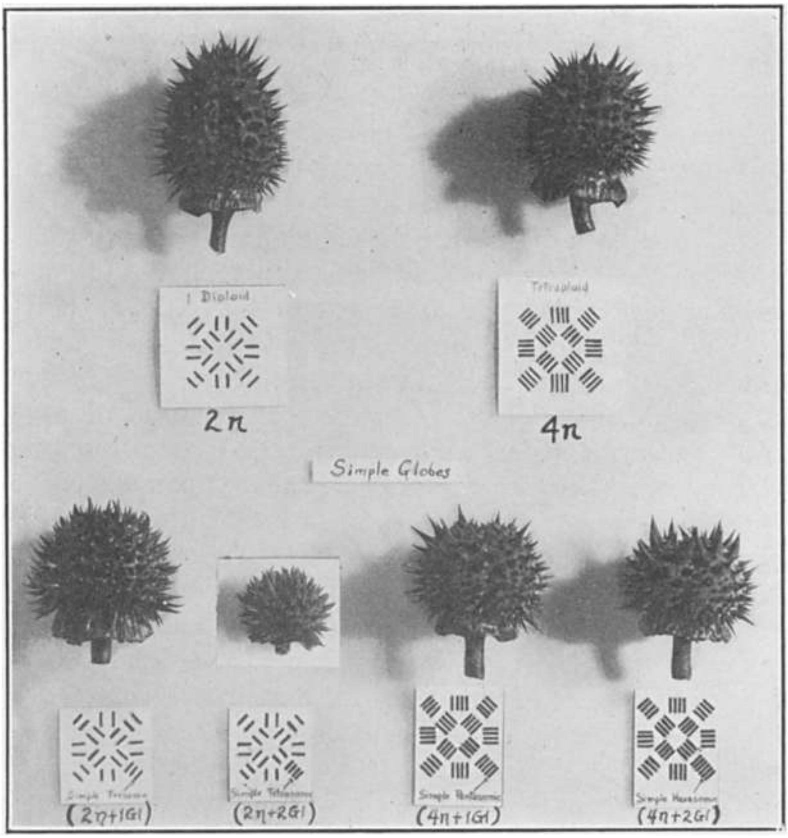

FIG. 7. Above, capsules with diagrams of a diploid (2*n*) and a tetraploid (4*n*). Below, capsules with diagrams of the different Globe mutants.

appearance, at least in the Globe series just discussed. In double mutants, moreover, somatic effects may be intensified or largely neutralized by individual genes in the two extra chromosomes, and an easy expression of the combined unbalance which they exert will therefore be impossible.

The structural characters have been taken for illustration from a particular part of a single mutant, the Globe. A more detailed study of changes in external and internal morphology brought about by the presence of specific extra chromosomes in the several mutants is being undertaken in cooperation with Dr. Sinnott.

The unbalancing effect of an extra chromosome is shown in the lessened vigor of mutant plants. Thus from Globe parents as an example of $(2n+1)$ mutants, ordinarily only one quarter of the offspring to reach recordable size are Globes, instead of the 50 per cent. expected. Moreover, when the plants are crowded the proportion of Globes surviving is considerably lessened.

We have been discussing the unbalance as affecting the sporophytic generation. In the gametophyte, the unbalance is doubled. Thus from $(2n+1)$ Globe plants with an unbalance of 1 over $2n$ the pollen grains with the extra chromosome have an unbalance of 1 over n. This extreme unbalance hinders their functioning and brings it about that the Globe character is transmitted to only a slight extent through the pollen (under 2 per cent. in a considerable series of crosses). It is of interest in this connection to note the results of selfing and crossing Globes of the tetraploid series. The unbalance in a $(4n+1)$ Globe is 1 over $4n$, while the unbalance in its pollen grains which carry the extra chromosome is 1 over $2n$. Due to this lessened unbalance in comparison with pollen of $(2n+1)$ Globes, the pollen of the $(4n+1)$ Globe transmits the Globe character to a higher percentage of its progeny (14 per cent. in the single pedigree tested), and partially for the same reason we have obtained higher proportions of Globes in the offspring from selfing such $(4n+1)$ Globes (a total of about 60 per cent. in a single experiment). A more specific study of the effect of extra chromosomes upon the gametophyte is being undertaken in cooperation with Dr. Buchholz.

It will not be advisable at the present stage of our investigations to discuss the possible external and internal factors which may induce the chromosomal aberrations which form the basis of our common mutations in *Datura*.

A study of the effects of radium rays undertaken in coöperation with Dr. Gager has given results which, although in an early stage of the experiment, appear suggestive in this connection. Other stimuli are being tested which appear to induce irregularities in the distribution of chromosomes to the pollen grains. It will be a matter of theoretical interest to be able to control experimentally the production of chromosomal mutations. It might also prove to be of considerable economic importance to be able to produce at will the full range of chromosomal mutants in any plants, especially in those which are propagated by vegetative means.

To us, one of the most interesting features of the *Datura* work is the possibility afforded of analyzing the influence of individual chromosomes upon both the morphology and physiology of the plant without waiting for gene mutations. Evidence is at hand which indicates that every chromosome in *Datura* carries factors which influence the expression of the so-called unit character purple pigmentation. Our work so far we believe adds evidence to the conclusion that the mature organism—plant or animal—is not a structure like a child's house of blocks, made up of separate unit characters, nor is it determined by separate and unrelated unit factors. It is rather the resultant of a whole series of interacting and more or less conflicting forces contained in the individual chromosomes.

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