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Triploid Mutants in Oenothera[1]).

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In a paper dealing with "The Stature and Chromosomes of *Oenothera gigas*, De Vries", published in 1909, Gates, in discussing the possible origin of the 28-chromosome condition in *O. gigas* made the following statement:

"De Vries describes the appearance of a mutation as resulting from the union of a 'mutated' germ cell with an ordinary germ cell. However this view can scarcely apply in this case, since, although it is possible that germ cells may occasionally be produced with the unreduced number of chromosomes, fertilization with such a germ cell would produce an organism with 21 instead of 28 chromosomes. The possibilities of two such unreduced germ cells — an egg and a sperm — getting together in fertilization are very remote. Moreover, no instances of this sort are known, and if this were the method of origin one would also expect to find mutants occurring with 21 chromosomes."

In reply to this statement, Stomps (21) in 1910, pointed out that it is not improbable that 21-chromosome mutants do occur in

1) Report of investigations conducted at the Station for Experimental Evolution. Cold Spring Harbor.

cultures of *Oenothera*. He called attention to the fact that no offspring had been grown from the two last plants of de Vries' culture which had been identified as *O. gigas*, and it was therefore possible that they were triploid, and not tetraploid mutants. He also stated that he agreed with Professor de Vries in the opinion that such „halve mutanten" have since been repeatedly derived from seeds of purely fertitized *O. Lamarckiana*.

Recently (1911), in referring to Stomps' (21) statements in regard to the possible origin of the tetraploid condition in *O. gigas* through the union of two „mutated" germ cells which had 14 chromosomes each, Gates says (15):

"If Stomps' theory were correct, we should have a mutant occurring with twenty-one chromosomes, and it would be much more frequent in occurrence than in *O. gigas*. Such a mutant has never yet been found, and all the other mutants which are known have fourteen chromosomes, as in *O. Lamarckiana*."

It is not the purpose of this paper to discuss the manner in which *O. gigas* arose. Such evidence as has been observed in the somatic cells of *Oenothera* bearing directly upon this subject will be fully stated and discussed in more detailed reports to follow. In view, however, of the above assertions by Gates and Stomps, it was thought desirable to offer some statement in advance concerning the appearance of a 21- and a 22-chromosome mutant among the pedigreed cultures of *Oenothera* at the Station for Experimental Evolution[2]).

In the summer of 1908, I recognized a new form (Fig. 3)[3]) among the *Lamarckiana* hybrid offspring of *O. lata* × *O. Lamarckiana* which was found, — from studies of root-tips in 1909, — to have 21 chromosomes (Fig. 1). Two individuals were observed in separate cultures of this cross. A close watch was kept for the reappearance of this form in 1909, but it was not recognized[4]). It

2) All the Cold Spring Harbor cultures to which I shall refer in this or succeeding papers may be understood to have descended through pedigreed cultures of guarded pollinations from plants or seeds from de Vries unless clearly stated to the contrary. Most of the somatic counts referred to in this paper were determined from sections of root-tips, but a few were obtained from sections of young buds. None were considered established unless clearly demonstrated beyond all possible question. In most cases these were determined from a large number of figures in metaphase distributed through 3 or more fixations from each plant.

3) This photograph is unfortunately very poor, but it will serve to illustrate several of the chief points of interest in connection with this mutant.

4) A culture of *O. nanella* × *O. gigas* was grown in the summer of 1909, and 46 offspring came to flower as annuals, a large number of which strongly resembled the offspring of *O. Lamarckiana* × *O. gigas* growing in the garden at the time. Two individuals, however, had many points in common with the 21-chromosome mutant. Somatic chromosomes of one of the two were studied and the number found to be in the region of 21, and probably 21. Inasmuch as this is

was again identified in 1910, however, making a total of 8 individuals for the two seasons as follows:

	Parentage	Cultural number	Year grown	Number of individuals
1.	*O. lata* (15)[5]) × *O. Lamarckiana* (14)	6024	1908	1
2.	„ „ „ „ „ „	6027	1908	1
3.	*O. Lamarckiana* (14) × *O. Lamarckiana* (14)	6046	1910	1
4.	„ „ „ „ „ „	6048	1910	3
5.	„ „ „ „ „ „	6051	1910	1
6.	*O. lata* (15) self-pollinated	5343	1910	1

The number of chromosomes of this new form was definitely determined in only three plants, Nos. 2, 5 and 6; root-tip fixations having fortunately been prepared from 2 and 6. The number of figures observed in which the chromosome number could be clearly ascertained, while not so abundant as in some forms I have studied, left no question as to the certainty of this count. Studies were made from young buds of each representative of No. 4, and the chromosome number appeared to be 21 in each case, though not definitely ascertained[6]). In no one of the 6 individuals did I find any indication of deviation from this number. The chromosomes of the two remaining plants were not examined.

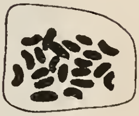

Fig. 1.

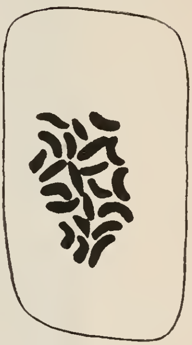

Fig. 2.

This mutant was not distinguished from *O. Lamarckiana* in the 1908 cultures until coming to flower, when it attracted the eye, standing among a group of *Lamarckianas*[7]), by the greater size of the buds and flowers, deeper yellow of the petals, and, late in the season, by the deeper red of the sepals. In 1910 it was recognized

the expectation for offspring of this cross, it proved nothing. Since no other mutants were recognized in this cross, and since the two offspring of *O. nanella* × *O. gigas* differed from the 21-chromosome mutant in a few points, it is probable that they were true hybrids. However, since the identity of the two plants was not clearly established, they must remain unclassified.

5) The numbers in parentheses indicate the chromosome number typical of the species, and not actual counts. Only *O. lata* of No. 6 was studied and determined.

6) It is usually far more difficult to determine chromosome numbers from the somatic cells of the young buds than from the root-tips, as the figures of the former are usually smaller and the chromosomes more crowded together and less clearly defined. When I relied solely upon bud fixations for these studies, it was a very common experience to fail to determine the count.

7) Following the precedent of the English translation of „Die Mutationstheorie" I shall form the plural of generic and specific names by the addition of *s*, but print in italics as for Latin words.

in the early rosette stage by the somewhat broader and more roundly pointed leaves, and in the late rosette stage in the garden was noted for the much increased diameter of the rosette and its apparent increased vigor. In height and general branching and flowering habits this form strongly resembled *O. Lamarckiana*, but suggested *O. gigas* in the increased size of the buds and flowers, the greater stoutness of the stem, branches and all parts of the plant,

Fig. 3.

and the deeper yellow of the petals. The sepal coloration late in the season was the deep red of certain types of *O. gigas* and certain other types of *Lamarckiana* mutants, though less intense than that of *O. rubrinervis*. The seed-capsules were longer than for *O. Lamarckiana*, though not so long as for *O. rubrinervis*. Each of the 8 individuals recognized came to flower as annuals, though slightly later than the majority of *Lamarckianas* surrounding them. A moderate abundance of pollen was produced by most buds, which was found to be composed of the common 3-lobed grain and grains having

4 or more lobes, though the former were much in excess of the latter. A much higher percentage lacked protoplasmic content than is typical for *O. Lamarckiana.* I daily self-pollinated one or more flowers of 5 of the 6 1910 mutants (several were also cross-pollinated) for two or three weeks during the height of the flowering season (and quite often the pollen appeared sufficiently good to insure fertilization), but only comparatively few seeds were harvested at the end of the season. These were grown by Dr. G. H. Shull[8]) (from whose cultures most of the fertilizations were made), in the summer of 1911.

The question will doubtless arise in the minds of many as to whether this so-called „mutant“[9]) having 21 chromosomes is truly a mutant or only a hybrid resulting from the accidental fertilization of *O. Lamarckiana* by *O. gigas* pollen, — because of the chromosome number and the combination of certain vegetative characters peculiar to these two forms; but that it is truly a mutant and not a hybrid of the latter cross is proven by the following facts:

I. The individuals composing this group were distinguished by the same chromosome number and the same vegetative characters whether derived from

a)[10]) *O. lata* (15) × *O. Lamarckiana* (14),
b) *O. Lamarckiana* (14) × *O. Lamarckiana* (14), or
c) *O. lata* (15) self-pollinated.

If we suppose that this form arose in each of the above cases through the accidental fertilization of the female parent by pollen from *O. gigas,* we must then conclude that these plants were hybrid offspring of *O. lata* × *O. gigas* in Nos. 1, 2 and 6, and hybrid offspring of *O. Lamarckiana* × *O. gigas* in Nos. 3, 4 and 5. Hybrids with 21 chromosomes do occur in each of these crosses, but so far as I have observed, they do not duplicate each other in vegetative character, nor have I found a 21-chromosome hybrid in either group which duplicated the vegetative characters of the 21-chromosome mutant.

II. *Oenothera gigas* was grown a quarter of a mile distant from

8) Dr. Shull kindly placed his cultures at my disposal for these studies of somatic chromosome number in relation to vegetative character during each of the four seasons following the beginning of the work in January, 1907. Although I grew my own cultures in 1909 and 1910, the studies of the first two years were made exclusively from Dr. Shull's cultures, and those of 1910, partly so.

9) The term „mutant“ is used throughout this paper and others to follow, in the de Vresian sense, and does not necessarily imply that the offspring of the plant in question will reproduce the characters of the parent throughout later generations.

10) It will be recalled that the chromosome number was determined for one triploid mutant offspring from each of these groups.

the *Lamarckiana* parents of 5 of the 6 1910 triploid mutants[11]) and operations upon these two groups were conducted by separate individuals. That *O. gigas* pollen should have accidentally reached the guarded stigmas of the 3 *Lamarckiana* parents of these mutants is therefore not probable. The *lata* parent of the 6th plant was, however, grown near the *gigas* cultures.

In 1910 a mutant of a type of character in some respects quite distinct from that of the form just described was observed in still another culture (6052) of *O. Lamarckiana* × *O. Lamarckiana*. As in the case of the 1910 21-chromosome mutants derived from the fertilization of *O. Lamarckiana* by *O. Lamarckiana*, *O. gigas* was grown a quarter of a mile distant from the *Lamarckiana* parents of the mutant in question, and operations upon the two groups were conducted by separate individuals, so it is here also improbable that the plant in question was derived from the accidental fertilization of the female parent with *O. gigas* pollen. The chromosome numbers of the parents were not determined; they were typical *Lamarckianas*, however, and it is therefore probable that the somatic chromosome number of each was 14.

In the greenhouse rosette stage, this plant, which I shall designate by its pedigree number 5589, was first described as being similar to *O. Lamarckiana*, though the center of the rosette appeared a little more compact, and the leaves were broader and more grayish

11) Although the actual number of chromosomes was determined in but 3 of the 8 mutants above referred to, the term "21-chromosome mutant" is here used merely as a temporary designation for all plants having the same vegetative characters as the 3 individuals which were found to have 21 chromosomes. Following papers will show that, so far as has been observed, all individuals which have the same vegetative characters throughout their life histories invariably have the same chromosome numbers. The exceedingly rare occurrence of a cell with a chromosome number varying from the normal by one, or having double that of the type — and two tips which showed certain irregularities that will be later described, — were practically the only exceptions noted in a total of 9441 tips and 186 buds studied. While it is a very common experience to find two plants with identical chromosome numbers differing conspicuously in their vegetative characters, I have never observed a single instance of two plants having the same vegetative characters in all stages of development, differing in chromosome number. Mention was made in one of my first reports (19) of several examples of supposed variation of chromosome number among individuals of certain species, but it will be shown in following reports that these supposed variants were demonstrated by later cultures to have been distinct forms.

The above assertions are based upon 5 years study of somatic chromosome number in the *Oenotheras*, 4 of which were in relation to vegetative character. Somatic chromosome number has been studied in 300 individuals (all but 8 of which were of known parentage and from guarded fertilizations) and exactly determined in 228 of this lot. All but 14 of the 300 were descended from seeds or plants from de Vries' and were grown at the Station for Experimental Evolution. 2 of the remainder were grown by Professor de Vries', and the remaining 12 by Dr. D. T. MacDougal at the New York Botanical Garden. The latter were derived from cultures of English ancestry.

pubescent. A little later it was again recorded as resembling *O. Lamarckiana*, though having a broader leaf and shorter petiole than *O. Lamarckiana*. On May 12 it was described for the first time as having many characters suggestive of *O. gigas*. It strongly resembled a plant from the latter culture which did not come to flower. On June 24 it was so badly attacked by aphids that it was difficult to describe, but the petioles had increased so much

Fig. 4.

in length that it no longer appeared to be *O. gigas*, though it still had many points suggestive of the latter form. At maturity however, *O. gigas* was again strongly suggested in the large size of the buds and flowers, and the deep yellow color of the petals, the stoutness of the stem, branches and all parts.

5589 differed from the 21-chromosome mutant chiefly in the manner of branching, which suggested *O. Lamarckiana* very little, if any at all. A comparison of photographs will show that the basal branches (4 in number) were much more rapidly ascending in 5589 (Fig. 4) than in *O. Lamarckiana* (Fig. 6), or the 21-chromosome mutant (Fig. 3), so that the distance between the tops of the farthest outreaching basal branches was much less than that for either of the latter. This is a character which is distinctive of most of the types of *O. gigas* having basal branches which I have observed (Fig. 7).

Fig. 5.

5589 differed further from the 21-chromosome mutant in that it maintained yellow buds throughout the season, though the bud-cones were angular and regularly tapering, as were those also of the 21-chromosome mutant. Pollen was produced in less abundance than in the latter form and was found also to be composed of a mixture of 3-lobed grains and grains having 4 or more lobes, but there appeared to have been even a higher percentage of grains lacking protoplasmic content than was characteristic of the 21-

chromosome mutant. Persistent effort was made throughout the flowering season to self-fertilize this plant, but less than a dozen seeds were obtained. *O. gigas* pollen, known to be capable of fertilizing flowers of the individual from which it was derived, was repeatedly applied to the stigmas of 5589, but with the same results obtained from the self-fertilization of this plant. It was then noted

Fig. 6.

that very few seeds had developed in the capsules of the open-pollinated flowers, showing that the female germ cells were mostly sterile, as this plant was surrounded by *Lamarckianas* in full flower[12]).

Unfortunately, it was utterly impossible to determine the exact number of somatic chromosomes in this form. It was ascertained

12) De Vries found a plant among his cultures of *O. gigas* in 1899 which failed to produce seed from artificial self-fertilization, but it is possible that this result was due to defective pollen, as suggested by Gates (15).

with certainty however, that there were not less than 20 nor more than 22.

The offspring of 5589 were also grown by Dr. Shull in the summer of 1911.

It was first thought that this plant might represent some type of *O. gigas*, possibly that of the original mutant, for it reached

Fig. 7.

a height of 12.2 dm. at the close of the season without having entirely completed flowering (and therefore without having reached its full height), and de Vries described *O. gigas* (5, Bd. I, p. 158) as attaining about the same height as that of *O. Lamarckiana*. *O. Lamarckiana* in the Cold Spring Harbor cultures commonly completed growth at a height of 13 or 14 dm. *O. gigas* of these cultures on the contrary, seldom attained a height of 12 dm., and commonly did not exceed 10, 8, or even 6 dm. However, it must be recalled that all *O. gigas* now in cultivation are probably descended from de Vries' 1895 mutant. Is it not therefore possible that the years of inbreeding which have followed have reduced the stature of this form and have altered other characters to some extent as well?

Further careful study, however, demonstated conclusively that 5589 was not *O. gigas*. The nodes were more widely separated, and while the stoutness of the plant was much increased over that of *O. Lamarckiana,* it was still less pronounced than that of *O. gigas*, and represented an approach towards, rather than a duplication of the *gigas* condition.

A third interesting mutant, 4453 (Fig. 5), appeared in a culture grown in 1909, derived from an *O. lata* mutant (known to have 15 chromosomes), self-pollinated.

The rosette leaves of this plant combined the *lata* crinkledness and general contour of leaf with the thickness of *O. gigas*. They were, however, less finely crinkled than is characteristic of *O. lata*. The adult foliage was distinguished in the same manner. It produced 10 basal branches, although all can not be counted in the photograph (Fig. 5), and completed flowering at a height of slightly less than 7 dm. The increased stoutness of the stem, branches and all parts of the plant was very noticeable here as for the 21-chromosome mutant, and mutant 5589. The shape of the bud, however, differed markedly from that of the two other forms. While the buds of 4453 were much larger than those of *O. lata*, the first had the irregular shape so characteristic of the first buds of *O. lata,* though later ones of both forms became more regular and tapering. Although the early buds had practically no red on the sepals, little fleckings appeared on the later ones, and by the close of the season those of the branches which had been flowering longest were covered with a fairly even thin red. This, however, was much less intense than the coloration of the buds of the 21-chromosome mutant. It will be recalled that those of 5589 remained yellow until the close of the season. The absence of red on the sepals is also characteristic of *O. lata.*

In *O. lata* the length of the calyx of the first buds is insufficient to accomodate the full growth of the petals and style without much compression. It is this condition that causes the irregular shape of the early buds in this form. When the flower expands, the petals are unfolded in a crumpled condition, and the style is frequently found to be bent into a single or double elbow. The crumpling of the petals in *O. lata* is one of the causes of the earlier fading, for, with the rising of the sun, the flowers of this plant are among the first in the garden to droop.

A condition similar to that just described for *O. lata* was found in the flowers of 4453 (which were much larger than those of *O. Lamarckiana* or *O. lata*), though somewhat less pronounced.

The petals were a slightly deeper yellow than is typical of the parental form (this appearance possibly being due to increased thickness of the petals), and the flowers resisted the effect of the morning sunlight a little longer than *O. lata*.

The anthers had much the same appearance as those of the parental form, and but very little pollen was exposed. However, by pursuing the same methods as those which will be later described for the fertilization of *O. lata*, enough pollen was secured to thoroughly cover the stigmas of 3 or 4 flowers. But 4 seeds were obtained, all of which failed to germinate.

From studies of root-tips from this plant the somatic chromosome number was found to be 22. Many beautiful figures were observed in which the chromosomes were well separated and clearly defined, leaving no question whatever as to the certainty of the count.

The nature of the vegetative characters and the somatic chromosome numbers of the offspring of the mutants above described become a subject of great interest and importance. Dr. Shull has kindly given me permission to quote from his descriptions of these forms in the early rosette stage (May 12, 1911).

Offspring of 1910 21-chromosome mutants (first form described in this report:

5445 self-fertilized (described above as No. 3, culture 6046) "produced only 3 plants, no two of which are alike; one conforms to the 5509[13]) type, and another resembles linear-leafed *gigas* with lanceolate, acute leaves, not crinkled, but with margins strongly undulate. The third also resembles *gigas* in some ways, but with broad, uncrinkled leaves."

5483, self-pollinated (one of the three individuals described above as No. 4, culture 6048), "has given me a family of 77, all but 2 of which are apparently good *O. Lamarckiana*. These 2 have round crinkled leaves resembling *O. lata*, tho a little darker green and having a heavier texture than usually seen in that form."

5558, self-pollinated (described as No. 5, culture 6051), "produced 8 offspring, 3 of which were *albida*, 1 *lata* and 1 having broad heavy leaves with winged petioles, grayish pubescent, somewhat resembling *O. gigas*. One other had long, lanceolate, sharp pointed, shining, darker green leaves, thick and leathery. The other two are too young to describe at present."

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13) 5509 represented a type of mutant frequently appearing among the cultures of *O. Lamarckiana* at Cold Spring Harbor which was thought to have many characters corresponding to de Vries' descriptions of *O. oblonga*. The frequency of its appearance seemed a further indication of its identity, but Professor de Vries has recently stated to me that so far as he could judge from photographs and descriptions, this form did not appear to be *oblonga*.

5484 × 5483 (two individuals of No. 4, culture 6048, crossed), "produced 7 offspring, no two of which were alike. These have been described as follows: 1. Stiff, ovate, uncrinkled, grayish leaves, spreading in centre of rosette. 2. Rather firm, crinkled leaves, broadly oblong, slightly apiculate, erect in centre of rosette. 3. Resembles *O. nanella*, but with oval, rounded leaves. 4. Leaves large, but with very broad crinkled blades and winged petioles. Grayish pubescent. 5. Resembles linear-leafed *gigas*. Leaves nearly horizontal, light green, slightly grayish, uncrinkled. 6. Similiar to 1, but leaves less acute, less grayish, and a little more ascending. 7. Broadly oblong, acutish, crinkled, grayish. Centre of rosette moderately erect, but large leaves nearly horizontal.

5558 × 5483 (No. 5, culture 6051 × one individual of No. 4, culture 6048 — same male used in the cross just described), "produced 7 offspring, no two of which were alike, but most show some resemblance to the several forms of *O. gigas*. These have been described as follows: 1. Thick, dark green, ovate leaves, almost uncrinkled. 2. Very crinkled, looks intermediate between *gigas* and *lata*. 3. slightly resembling *O. Lamarckiana*, but with darker green leaves of thicker, fleshier texture. 4. Could easily be classed as a moderately broad-leafed *gigas*. 7. Rather young, but appears now similar to *O. Lamarckiana* of same age."

Nos. 5 and 6 were not characterized. They were probably too young.

The second mutant, 5589 (having a chromosome number in the region of 21), self-pollinated, produced but one offspring of the 5509 type mentioned among the progeny of 5445.

The offspring of 5589 × *O. gigas* need not be considered here.

As was previously stated, no germinations were obtained from the seeds of the 22-chromosome mutant 4453.

From the fore-going it is clear that the 21-chromosome mutant does not breed true with respect to vegetative character. Unfortunately, nothing is known of the chromosome numbers of these plants, as the opportunity for this study was not afforded. While too much weight must not be attached to speculations in regard to the chromosome numbers of these offspring, some statement as to the probabilities may not be amiss.

So far as I have observed (as previously stated), I have found that all individuals of a given type of vegetative character invariably have identical somatic chromosome numbers, regardless of the diversity of origin of the individuals in question. If the 75 offspring of 5483 self-pollinated which were 'apparently good *O. Lamarckiana*' in the early rosette stage maintained these characters throughout their life histories, and were in every way indistinguishable from *O. Lamarckiana*, I think it is probable that each had 14 chromosomes.

All individuals which I have observed having a chromosome number much in excess of that of *O. Lamarckiana* displayed certain characters strongly suggesting those of *O. gigas*, chiefly noted in the stoutness of all parts. This was true of both mutants and hybrids, and the only exceptions noted were those of certain second generation hybrids to be later described which maintained a high chromosome number in spite of their weak and sickly appearance. Even these retained the large buds and flowers of *O. gigas*. We may therefore assume that the possession of *gigas*-like vegetative characters by any form is an indication (not a proof) of a chromosome number considerably in excess of that of *O. Lamarckiana*[14]).

On the basis of this assumption it would appear that 80 or more of the above described 98 offspring of the 21-chromosome mutants had a somatic chromosome number of 14, or in the region of 14 (*lata*, *albida* and the 5509 type), but that most of the remainder approached or equalled that of the parents, or of *O. gigas*.

This is indeed very suggestive of irregularities in the divisions of the germ cells of the 21-chromosome mutants. In this connection the report of Geerts (16) upon the reduction division of the germ cells of a 21-chromosome offspring of *O. lata* × *O. gigas* is of especial interest:

„In unseren *Oenothera*-Bastarden treten die Chromosomen aus dem Knäuel in der vegetativen Zahl hervor, also 21 (Fig. 1). Nach der Auflösung der Kernmembran zeigen sie den Anfang der paarweisen Anordnung, aber es entstehen nur 7 Paare, während 7 Chromosomen ungepaart bleiben. Bei der ersten Teilung, bei der Reduktionsteilung, trennen sich von den 7 Chromosomenpaaren ganze Chromosomen voneinander, welche zu je einem Pol gehen (Fig. 2).

14) It should be stated in the connection that mutants with low chromosome numbers have been found with relatively stout stems and thick buds, so that the increased stoutness of certain parts is not an invariable indication of increased chromosome number. This condition, however, was confined to forms of low stature, and to individuals which resembled *O. gigas* in no other parlicular.

Gates (13), in discussing the offspring of *O. lata* × *O. gigas*, mentions the possibility of an individual having the tetraploid number of chromosomes owing its *gigas* characters to the *number* of chromosomes rather than their *source*. He also brought out evidence to show, form a series of measurements, that certain cells of the tetraploid mutant *O. gigas* are conspicuously larger than those of *O. Lamarckiana* having the diploid number, and states that the larger cells doubtless account for the greater stature of this plant (12).

While I think it is probable that the increased stoutness of *O. gigas*, and perhaps certain other characters as well, are the direct result of the doubling of chromosome number and increase in cell dimensions, I believe, as Stomps has stated (21, p. 63), that it is very improbable that all of the characters of this mutant are the direct expression of this increase. It is certain that the larger cells of *O. gigas* are not responsible for increased height in this plant, for the *height* is not increased. As previously stated, de Vries described *O. gigas* as attaining about the same height as that of *O. Lamarckiana*.

Von den 7 gesonderten Chromosomen wandern gewöhnlich 3 nach dem einen, 4 nach dem anderen Pole (Fig. 2), bisweilen liegen sie unregelmäßig in der Spindel zerstreut oder sind vielfach zerteilt (Fig. 3 b). Zu jedem Pole gehen also 7 Chromosomen, welche, wenn sie dem Pole etwas mehr genähert sind, schon die Längsspaltung für die zweite Teilung als eine mehr oder weniger tiefe Einschnürung aufweisen und 3 oder 4 Chromosomen, welche meistens unregelmäßiger und weniger deutlich gespalten sind. Bisweilen erreichen sie die Pole nicht und treten also, wenn sich um jeden Kern eine Wand ausbildet, nicht in die Kerne ein."

„Bei der zweiten Teilung werden 7 deutlich gespaltene Chromosomen in die Kernplatte eingereiht und 3 oder 4 kleinere, welche entweder eine mehr oder weniger tiefe Einschnürung zeigen oder sehr unregelmäßig gebildet sind (Fig. 5 und 6). In jeder Spindel lösen sich also von 7 Chromosomen die Hälften voneinander und wandern nach dem Pole, während die anderen Chromosomen in der Spindel verteilt liegen oder in Stückchen zerfallen. Zu den 4 Polen gehen also immer 7 deutliche Chromosomen und oft eine Zahl unregelmäßiger Chromosomen oder Chromatinstückchen [15])."

The above condition was described for the type first reported by de Vries (6) as intermediate between *O. lata* and *O. gigas*, and designated by Geerts as the "*lata*-Typus". He found a similar condition in the other hybrid common in this cross which he calls "*gigas*-Typus", and which was first described by de Vries as having the appearance of combining *Lamarckiana-gigas* characters (6). Furthermore, he found the reduction divisions in the two hybrids *O. gigas* × *O. Lamarckiana* and *O. Lamarckiana* × *O. gigas* to agree in every respect with those ot the "*lata*-Typus" from *O. lata* × *O. gigas*.

Of further interest is the fact that he found 14 chromosomes in one individual of the second generation of *O. gigas* × *O. Lamarckiana*. He also states that the second generation offspring of *O. gigas* × *O. Lamarckiana* showed the characteristics of *O. gigas* quite as well as did those of the first generation. I have determined the somatic chromosome number approximately in 53 second generation hybrids (49 *O. lata* × *O. gigas* and 4 *O. Lamarckiana* × *O. gigas*)

15) Stomps (21) had anticipated a condition in the reduction divisions of 21-chromosome plants somewhat similiar to that later observed by Geerts for these hybrids. Gates had reported that all of the 21 chromosomes of a triploid offspring of *O. lata* × *O. gigas* were distributed to the two poles of the spindle at reduction in this plant, ordinarily in groups of 10 and 11. Stomps, in discussing these results expressed the opinion that this condition was analogous to that described by Rosenberg for *Drosera obovata*, and that 7 pairs were formed, while the remaining 7 chromosomes were distributed to the two poles, ordinarily in groups of 3 and 4 each.

and precisely in 42 of this number. Some were observed to have low, some high chromosome numbers, but not a single instance was found in which *gigas*-like vegetative characters were associated with a low chromosome number, although many of these second generation offspring resembled *O. gigas* quite as pronouncedly as have any 21-chromosome hybrids of the first generation which I have thus far observed. However, but 12 of the 53 were offspring of 21-chromosome plants. These hybrids will be described in following papers.

Gates (8, 9, 10) was the first to call attention to irregularities in the heterotypic divisions of the germ cells of *Oenotheras*, and these observations were recorded from plants with 20 and 21 chromosomes respectively, which he later believed to have been offspring from *O. lata* × *O. gigas*. In these plants he found that one chromosome occasionally passed to the wrong pole of the spindle in the reduction division of the male, resulting in the distribution of chromosomes in groups of 9 and 11 in the former case and 9 and 12 in the latter. He also observed a similar condition in the *Lamarckiana* hybrids of *O. lata* × *O. Lamarckiana* (9, 11). This irregularity in chromosome distribution he later found to occur occasionally in the reduction division of the male germ cells of *O. gigas* and other forms. He recorded a single instance in *O. gigas* (15, p. 923) in which a pair of homotypic spindles in late prophase showed 12 and 16 chromosomes respectively, indicating, as he says, that a 12—16 distribution of chromosomes had occurred at heterotypy. He states that this is the widest departure from an equal distribution of chromosomes that he has found.

Gates observed a second type of irregularity in the distribution of chromosomes in the heterotypic and homotypic divisions of various forms which is quite as significant as the first. He found many instances in which a single chromosome had been left behind during reduction, and had formed a small supernumerary nucleus (13, p. 184). This occurrence was noted particularly in hybrids. He mentions a case of especial interest in *O. gigas* (12, p. 528). Here he found three chromosomes left behind in the cytoplasm after the formation of the daughter nuclei at the end of reduction. He does not state, however, whether all belonged to one nucleus, or two to one, and one to another.

The condition which Gates found at the end of the homotypic division of the male germ cells of *O. lata* (7, p. 98) indicates that numerous irregularities in chromosome distribution occur in the maturation divisions of the male germ cells of this form. He found the normal number of four nuclei in some cases, but these sometimes varied in size and chromatin content. Occasionally extra nuclei were observed, and again others that were large and irregularly shaped; at other times a small extra nucleus with a single chromosome, or two small extra nuclei, each containing two chromosomes, etc.

It would be very instructive to know whether such irregularities may be found at this stage in the female germ cells of *O. lata*, or whether this phenomenon is connected especially with the sterility of the male.

Irregularities at this stage were occasionally found in the male germ cells of other forms, but apparently in no such abundance as in *O. lata*. For example, Gates recorded one case in *O. rubrinervis* (11, p. 18) in which two small nuclei were present in addition to the four larger ones composing the tetrad. He also mentions and figures a case in the telophase of the homotypic mitosis of a 21-chromosome plant (13, p. 188) in which one chromosome was left behind in the cytoplasm.

These conditions recorded by Gates are very suggestive of irregularities in the homotypic division in certain cases, and are of especial interest, taken in connection with Davis' statement in regard to *O. Lamarckiana* (4, p. 952):

"Thus, during the homotypic mitosis it is not uncommon to find that some of the chromosomes in a group of seven have failed to reach the poles of the spindle, and as a result form smaller supernumerary nuclei in the pollen mother-cell. Such a case is shown in Pl. LXXII, Fig. 44, where the chromosomes of three groups, a total of twenty-one, are distributed among five nuclei. Tetrads may even be formed in which large and small nuclei become associated in the same cell and pass into a resting condition, but it is not known whether such a cell can nature into a fertile pollen-grain."

It is very probable indeed that some of these various aspects observed at the end of the homotypic mitosis are the result of irregularities of the last division. The case recorded by Gates in which one chromosome was found to have been left behind in the cytoplasm after the formation of the daughter nuclei in the telophase of the homotypic division of a 21-chromosome plant strongly indicates that *it may be possible for a male germ cell to be finally equipped with less than the normal number of chromosomes, though fully supplied at reduction.* The question remaining is whether germ cells are functional when supplied with less than the normal number of chromosomes.

Davis (3, p. 638) reported having observed two cases in *O. biennis*, and recently (4, p. 949) a single case in *O. Lamarckiana* 'that showed numerical irregularities in the distribution of the chromosomes by the heterotypic mitosis'. He also refers to but one chromosome passing to the wrong pole of the spindle in these instances. However, he mentions a very interesting irregularity in the heterotypic mitosis of *O. gigas* of the same nature as the second type referred to by Gates. He says (4, p. 957):

"In this case two pairs of chromosomes had lagged behind the main group in passing to the poles of the spindle, and were associated in a small secondary spindle within the main structure. That such stray chromosomes, as well as the main group with a chromosome content smaller than the normal, may form independent nuclei is illustrated in Fig. 70. It is, however, improbable that fertile pollen-grains can be developed under such abnormal conditions. The fact that the chromosomes in these nuclei have lost their usual form and by anastomosing appear to be developing a chromatic reticulum, indicates that the nuclei do not proceed further towards the homotypic mitosis, but probably pass into a resting condition."

If the 75 offspring of the Cold Spring Harbor 21-chromosome mutant (5483) persisted in their *Lamarckiana* characters throughout their life histories (and it is practically certain that the majority did if they bore no other than *Lamarckiana* characters throughout the greenhouse rosette stage [16]), it would indicate that these offspring were the result of such irregularities in the heterotypic divisions of the germ cells of the parent as were described by Geerts for the germ cells of 21-chromosome hybrids. Furthermore, the presence of 75 such offspring in a total of 98 described would indicate that this phenomenon is of very common occurrence.

However, from the fact that a few of the offspring of these mutants were described as having vegetative characters resembling those of *O. gigas* more or less in the early rosette stage, I believe that we shall find, with more extended studies, that occasionally numbers of normal chromosomes in excess of 14 (perhaps ranging all the way from 14 to 21) are distributed to the poles of the spindle at the reduction division of 21-chromosome plants [17]. The two poles in such cases might receive equal or unequal numbers of chromosomes. It is obvious that one must necessarily receive one more than the other when the total number reaching the poles is 15, 17, 19, or 21. From this irregular distribution, I believe that germ cells sometimes result with a number of *normal* chromosomes in excess of 7. Let us assume that all of the 21 chromosomes occasionally reach

16) A recent communication from Dr. Shull states that 73 of the 75 offspring displayed no other than *Lamarckiana* characters throughout the season. Of the remaining two, one, at least, showed certain points of resemblance to *O. gigas*.

17) The second paragraph quoted from Geerts on p. 399 of this report clearly shows that a number of chromosomes (or remnants of chromosomes) in excess of 14 are sometimes distributed to the poles of the spindle at the reduction division ("*lata*-Typus" from *O. lata* × *O. gigas*).

the poles, and that the divisions are regular as described by Gates for a 21-chromosome plant, with only an occasional exception (such as he has noted in this form) of one chromosome passing to the wrong pole. If all remain normal, we should then have germ cells resulting as Gates has indicated, with 10 and 11 chromosomes commonly, and 9 and 12 exceptionally. These may so combine as to produce commonly, offspring with 20, 21 and 22 chromosomes respectively; and offspring with 18, 19, 23 and 24 chromosomes respectively, occasionally (provided of course that functional germ cells may result from an irregular distribution of chromosomes). According to expectations, 21-chromosome plants should occur most frequently among these offspring, and 18- and 24-chromosome plants most rarely. It does not necessarily follow that each of the former should duplicate the vegetative characters of the 21-chromosome parent, nor that all of these offspring should conform to one type.

If we assume that chromosome numbers much in excess of that of *O. Lamarckiana* are always associated with vegetative characters which suggest certain vegetative characters of *O. gigas* more or less, the above numbers (18, 19, 20, 21, 22, 23 and 24) would be sufficient to account for most of the offspring of the 21-chromosome mutants which were recorded as having vegetative characters resembling *O. gigas* more or less; but they could not account for the large number of *Lamarckianas* among the offspring of one of these mutants. Neither would Geerts' observations alone explain the presence of certain *gigas*-like characters in others, unless, as he states (16, p. 163) for the second generation offspring of *O. gigas* × *O. Lamarckiana*, a 14-chromosome offspring of a 21-chromosome plant may resemble *O. gigas* as strongly as did the parent.

If, however, we consider the possibility of irregularities of the second type observed by Gates and Davis (in which one or more apparently normal chromosomes fail to be included within either daughter-nucleus at the heterotypic or homotypic division of the germ cells), then we might easily complete the series from 24 to 14. However, it would be surprising to find that this last irregularity is of sufficiently common occurrence for a self-pollinated 21-chromosome plant (5483) to produce over 70 fourteen-chromosome offspring in a total of 77. If these *Lamarckiana* offspring had each 14 chromosomes, as we may assume with a fair degree of certainty, I think it is probable that this number was derived in each case from the fertilization of a 7-chromosome egg by a 7-chromosome sperm, and that these germ cells were produced in the manner described by Geerts.

On the basis of the nature of the vegetative characters of the offspring of the Cold Spring Harbor 21-chromosome mutants, the following suggestions may be offered as working hypotheses:

1. The heterotypic mitosis of 21-chromosome mutants and hybrids may sometimes distribute but 14 chromosomes, — which are destined to remain normal bodies, — to the two poles of the spindle. This distribution may be in groups of 7 each, ordinarily, but possibly occasionally in groups of 6 and 8 each.

2. The reduction division may sometimes distribute all of the 21 chromosomes to the two poles of the spindle. This distribution may occur ordinarily in groups of 10 and 11, but occasionally in groups of 9 and 12. All may remain normal bodies, or a few may afterwards degenerate.

3. A number of chromosomes anywhere between 14 and 21 may sometimes be distributed to the two poles of the spindle at the reduction division. The two groups may be numerically equal in such cases (approximately so when the number is odd), or unequal. All may remain normal bodies, or a few may afterwards degenerate.

It is assumed for 1, 2 and 3 that 14 of the original 21 chromosomes present at metaphase of the heterotypic mitosis will remain normal bodies.

4. The failure of one or more chromosomes to be included within a daughter-nucleus at the telophase of the homotypic division may result in the production of a germ cell with a chromosome number less than that of the number received at the heterotypic division preceding. The number may also be reduced, as indicated above, by the degeneration of one or more of the chromosomes received by the daughter-nucleus at the telophase of the heterotypic mitosis. Consequently, the somatic chromosome number of an offspring of a 21-chromosome plant does not necessarily represent the sum of the numbers received by the two parental germ cells at the heterotypic division.

The evidence does not indicate that we shall find one type of reduction exclusively in 21-chromosome mutant A, for example, and another in a sister mutant B. It is possible, in accordance with de Vries' experiments upon the variation of the 'Erbzahlen' in relation to the different positions of the fruits on the plant (5, Bd. II, pp. 414—418), that the type of reduction present in the male and female germ cells of a flower depends upon its position on the plant. We may find, therefore, that a single triploid mutant or hybrid may be represented by all types enumerated under 1, 2 and 3, but that each may be restricted chiefly to particular portions of the plant. For instance, the reduction division in both the male and female germ cells of the first flowers of a triploid plant might be represented by the Gates type almost exclusively, while that of the late flowers on the same branches (or stem) might exhibit chiefly the Geerts type of reduction, or vice versa. Perhaps also, the first flowers of a weak lateral or sub-lateral branch may differ from the first flowers of the stem or a strong basal branch.

The indiscriminate pollination of flowers without regard to the position of the pistil- or pollen-flower on the plant, — such as was practiced in the self- and cross-fertilization of the triploid mutants, — might very well result in the production of offspring with just such a variety of somatic chromosome numbers as that which is indicated by the vegetative characters of the offspring of these mutants.

The discrepancy in the observations of Gates and Geerts may have resulted from material having been fixed from early buds in one case, and from late in the other[18]. ("Early" and "late" of course refers to the first and last buds on the stem and branches, and not to the time of the season at which they were produced).

These questions can be settled only by extensive studies of the reduction division in the germ cells of flowers from various regions of 21-chromosome plants in connection with the exact determination of somatic chromosome number in a large number of offspring of these plants, self-pollinated. However, it is exceedingly difficult to obtain seed from the self-pollination of 21-chromosome plants, or the pollination of such an individual by another of its kind, and it may not be possible to obtain large cultures of such offspring

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18) I have since had the privilege of discussing this question with Dr. Geerts, and he tells me that his fixations from the 21-chromosome hybrids were made in September and October, and that they were taken from seed-plants, therefore from individuals which had produced their first flowers much earlier in the season. This indicates that Geerts' type of reduction appears in the later flowers, and Gates' probably in the earlier ones. Pollination of the Cold Spring Harbor 21-chromosome mutants was continued from the appearance of the first flowers (dates unrecorded, probably about the last of July) until the end of the second or third week in August. In our climate, seeds will not ordinarily ripen from later fertilizations. It therefore appears that the Geerts type of reduction may occur comparatively early in the season, though perhaps in flowers of a stem or branch which has been blooming for some time. It was my custom, in pollinating these 21-chromosome mutants, to cover the stigma of one flower with pollen from another. Occasionally, however, the pollen of a flower was applied to its own stigma. If pollen chanced to be taken from an "early" flower, and to be applied to the stigma of a "late" flower, then a 7-chromosome egg might be fertilized by a 10- or 11-chromosome sperm, and produce a 17- or an 18-chromosome offspring. Likewise, the fertilization of a "late" by a "late" might produce only 14-chromosome offspring. All the *Lamarckiana* offspring of 5483 may have been derived from the seeds of one capsule, and from the fertilization of a "late" flower with its own pollen, or with that of another "late" flower. Even though it may not be possible to ripen seeds from late fertilizations, it will be of great interest to determine whether they are more commonly produced from the pollination of "late" than "early" flowers of 21-chromosome plants.

Geerts' discovery of the degeneration of a portion of the chromosomes during the maturation process of certain triploid hybrids is a most important and significant one. A large field of inquiry is opened up in this connection.

in the immediate future. Since the chromosome numbers of the offspring of the Cold Spring Harbor 21-chromosome mutants are unknown, these plants, unfortunately, can not be accepted as positive evidence of one sort or another.

The discovery of 21- and 22-chromosome mutants opens the question of the identity of certain plants first described by Gates as offspring of *O. lata* × *O. Lamarckiana* (7). He said (p. 83):

"My garden of 1906 contained fifteen plants from seed of *O. lata* pollinated by *O. Lamarckiana*, ten of which conformed more or less completely to the characters of the pollen parent and four to the *lata* type, which is easily distinguished even in the rosette stage. One plant (no. 79), however, differed markedly from either of these forms, and was clearly a 'mosaic' hybrid, *i. e.*, in some characters it resembled one parent and in some characters the other parent. The petals, however, were considerably larger than those of either parent species, and the sepals showed streaks of red, suggesting the sepals of *O. rubrinervis* but much paler. This character, however, is common to all the plants of *O. lata* × *O. Lamarckiana* having the *Lamarckiana* characters, and is occasionally seen to a less degree in *O. Lamarckiana* itself. The large ovaries and stout hypanthium, the greater pubescence on the young buds, and the broad leaves with their obtuse tips, are all characters of *O. lata.* But the leaves were scarcely at all crinkled (the more or less complete absence of crinkling being a character of *O. Lamarckiana* which distinguishes it easily from *O. lata*), and the general habit of branching and greater luxuriance of growth also correspond with *O. Lamarckiana.* This plant is mentioned as showing that segregation of the parental characters is not always complete in this cross, as this individual was fairly intermediate in position between the two parents, though no fractionization of any characters was observed."

Gates' description of the so-called "'mosaic' hybrid" of this culture so closely resembles the description of the 21-chromosome Cold Spring Harbor mutant that I believe them to be the same thing. While he states that 'the large ovaries and stout hypanthium, the greater pubescence on the young buds, and the broad leaves with their obtuse tips are all characters of *O. lata*', the first three characters might be stated equally well as descriptive of *O. gigas*, and the last as distinctive of certain types of *O. gigas* (the linear-leafed form of course being a notable exception).

Unfortunately, Gates did not state the chromosome number of this plant, and it is probable that it was not ascertained.

In discussing the chromosome numbers of certain individuals of this culture, he said (p. 97):

"Several of the figures (figs. 37, 38, 39) show that the number of chromosomes in the *O. Lamarckiana* hybrid is greater than in *O. lata*. A count has not been made in the prophase, but from numerous counts in the metaphases and anaphases, the sporophyte number is found to be at least twenty and probably very near that number (fig. 37). It will be remembered that the sporophyte number in *O. lata* is 14"[19]).

He did not here state the number of *Lamarckiana* hybrids in which he found at least 20 chromosomes, but in a paper published in July, 1907 (8, pp. 10—11), in which the offspring of this cross are again discussed, he said:

"It is doubtless true that chromosome counts as ordinarly made come from a very few individuals. In the case of the *Lamarckiana* hybrid I have examined material from two individuals and from different flowers in each; these plants were grown in different seasons. In every count the number of chromosomes in the somatic tissues has been twenty or twenty-one, though I have been as yet unable to determine with certainly between these two numbers."

In the summary on page 20 he said:

"The number of chromosomes is now found to be twenty or twenty-one constantly in all the plants of the *Lamarckiana* hybrid examined, while the number in the pure *O. Lamarckiana*, as well as in *O. lata*, is fourteen, the reduced number being seven. ... Thus the pure *O. Lamarckiana* and the *Lamarckiana* hybrid, which are identical in external appearance, differ widely in the number of their chromosomes."

In again referring to this supposed culture of *O. lata* × *O. Lamarckiana* in a short paper (10) published in January, 1908, he said:

"*O. lata* from a cross was found to have 14 chromosomes as sporophyte number, but quite unexpectedly one of the other plants from what was believed to be pure seeds of *O. lata* × *O. Lamarckiana* was found to contain 20 chromosomes."

Speaking of irregularities of chromosome distribution in the heterotypic mitosis he said:

"Such irregularities will probably also explain other variations of one or two in the chromosome numbers, including the 20 instead of 21 chromosomes in what I have now shown to be an *O. lata* × *O. gigas* hybrid."

On the next page in the same note:

"I have also recently examined material from another plant in the cross which was believed to be pure *O. lata* × *O. Lamarckiana*,

19) Notwithstanding my first statements in regard to chromosome numbers in *O. lata* (see foot-note 11), it will be later shown that the specific number for this form is 15.

but which appeared to have all the characters of *O. gigas* and was found to have 21 chromosomes. This, with other evidence, furnishes definite proof that the cross which was believed to be pure had been partly pollinated with *O. gigas*"[20]).

And in conclusion:

"*O. gigas* has 28 chromosomes, and certain plants appearing in the first hybrid generation of *O. lata* × *O. gigas* having almost or quite the identical appearance of *O. gigas* have 21 chromosomes (20 in one plant), the latter segregating equally in reduction," etc.

In still another paper entitled „The Behavior of Chromosomes in *O. lata* × *O. gigas*" published in September, 1909, he states as a preliminary to the cytological discussions which follow:

"The plants from which these studies were made were grown at Wood's Hole, Mass., in 1905 and 1906, from seeds of De Vries. The results show that in some cases the number of chromosomes is undoubtedly 21, while in one individual it was 20[2]. The number is undoubtedly constant in an individual, however, as shown by a large number of counts, which demonstrated constantly 20 in one case and 21 in the other".

"The external characters were not studied with sufficient care at that time to describe them accurately, but from my notes they appear to have been intermediate between *O. lata* and *O. gigas*."

And in the note referred to:

"[2]In my first paper (8) this individual was thought to be *O. lata* × *O. Lamarckiana*, but was afterwards found to be derived from fertilization by foreign *gigas* pollen, this particular seed package not having been guarded as was supposed when the seeds were planted"[21]).

The evidence is not clear concerning the number of "*Lamarckiana* hybrids" studied, for there is nothing to indicate whether the 21-chromosome plant referred to in "The Chromosomes of *Oenothera*" was one of the two mentioned in the previous report (8, p. 10) or whether it was a third individual. Since he states, as quoted above, that the two were grown in different seasons, it is

20) This statement, together with the use of the term "foreign *gigas* pollen" in referring (13, p. 180) to the origin of the 20-chromosome plant of this culture, precludes the possibility of these plants having arisen from a sowing of mixed seed, a portion of which had been derived from the guarded fertilization of *O. lata* × *O. Lamarckiana* and the remainder from the guarded fertilization of *O. lata* by *O. gigas*.

21) This plant was mentioned as an offspring of *O. lata* × *O. gigas* in two papers published by Gates (11, p. 28; 12, p. 540), without any statement concerning its uncertain origin in either case other than that which is contained in the paper to which the reader is referred in the second publication. Such statements are certainly very misleading.

not known whether these two were the 20- and 21-chromosome plants, the 20-chromosome plant and another whose number was 'twenty or twenty-one' (8, p. 11), or the latter and the 21-chromosome plant. Since Gates has nowhere stated that these two or three plants were the offspring of separate crosses, it is probable that all were derived from the same lot of seeds, though grown in separate seasons. However, it is perfectly clear from the references which he mentions that the 20-chromosome plant referred to in each of these reports above quoted is the same as that first described as "*Lamarckiana* hybrid" offspring of *O. lata* × *O. Lamarckiana* (7). This plant has been so variously classified that the reader is left with no definite knowledge of the real nature of its vegetative characters. It was first described (7, p. 83) as belonging to a group of 10 plants 'which conformed more or less completely to the characters of the pollen parent' (*O. Lamarckiana*) and was called "*O. Lamarckiana* hybrid". Next (8, p. 20) it is stated that the pure *O. Lamarckiana* and the "*O. Lamarckiana* hybrid" (which we have seen includes the 20-chromosome plant) 'are identical in external appearance'. In the third paper (10) the 20-chromosome plant was included in a group of offspring of *O. lata* × *O. gigas* described as 'having almost or quite the identical appearance of *O. gigas*'. Lastly (13), it was mentioned in a group of hybrids which 'appeared to have been intermediate between *O. lata* and *O. gigas*'.

In the foot-note (13, p. 180) quoted above in which Gates described the origin of the 20-chromosome plant, the last statement is not entirely clear. It is probable that he intended to state that the fertilization of the plant from which the package of seed was obtained, was unguarded. This is indicated by the use of the term "foreign *gigas* pollen". To accept his meaning as stated would throw both parents in question. If, however, it was his intention to convey the thought suggested, then the question arises as to how one can know that the unguarded fertilization of any form was effected by *O. gigas* pollen. Even though there were no primroses growing in the garden except *O. lata* and *O. gigas* at the time fertilization occurred, it could be assumed only as a probability, for *O. lata*, whose anthers are usually dry and barren, occasionally produces a tiny bit of pollen which is capable of fertilization. In evidence of this fact, I have grown six cultures of *O. lata* at Cold Spring Harbor from six separate individuals of *O. lata* self-pollinated. This was accomplished only by gathering all of the unsplit buds from a number of individuals in the morning of the flowering day for days or weeks as necessary. These were then carefully opened in the laboratory, and when, rarely, a tiny bit of pollen was found, it was used to self-pollinate a guarded flower of the plant from which it was derived.

If anthers of *O. lata* are carefully examined with a hand lens it will usually be found that they have not opened and exposed such pollen as has been produced. By teasing them open with needles, it will be seen that they are partially filled with dry, shriveled grains lacking protoplasmic content. Scattered through this mass one can usually find a few grains which appear normal and healthy. If the number is increased to any considerable extent, the swelling of the large grains forces the anthers open in normal fashion, and the pollen is then exposed. Sometimes an anther is thus opened throughout its entire length, and sometimes only at the place where the bunch of normal pollen has been produced. One can not therefore say, no matter how barren the anthers of a plant may appear upon occasional examination, that it does not, very rarely, produce a tiny bit of pollen. Since a 21-chromosome mutant appeared in a Cold Spring Harbor culture of *O. lata* self-pollinated, having vegetative characters resembling *O. Lamarckiana* in certain respects and *O. gigas* in others, and a 22-chromosome mutant having vegetative characters strongly suggesting intermediacy between *O. lata* and *O. gigas* appeared in another culture of *O. lata* self-pollinated, it is not impossible that one or more of the plants of Gates' culture derived from the open pollination of *O. lata* and assumed to be offspring of *O lata* × *O. gigas*, were mutant offspring of *O. lata* fertilized with *O. lata* pollen.

If the *O. lata* parent of the 21-chromosome plant referred to in "Chromosomes of *Oenothera*" (10) was grown in the vicinity of *O. Lamarckiana*, for example, there would be no more occasion for assuming that this plant was derived from the fertilization of *O. lata* by *O. gigas* pollen than by *O. Lamarckiana* pollen, for a mutant is now known to have arisen from *O. lata* × *O. Lamarckiana* having 21-chromosomes and many vegetative characters suggestive of *O. gigas*.

While it is therefore possible, and quite probable that the 20- and 21-chromosome plants of unknown male parentage originated through the fertilization of the *O. lata* parent by *O. gigas*, as assumed by Gates, there is no means of proving it, either from the evidence of chromosome number or vegetative character; nor can we with any more security assume that they are mutants.

In his paper on "The Chromosomes of *Oenothera*" (10) Gates states, as previously quoted, that another plant in this cross which was believed to have been pure *O. lata* × *O. Lamarckiana*, but which appeared to have all the characters of *O. gigas*, was found to have 21 chromosomes. Since the 20-chromosome plant derived from the unguarded seed-package was then encorporated in his report (13) upon the offspring of *O. lata* × *O. gigas*, the reader is left to wonder whether the 21-chromosome plant was not similarly included, and the 20- and 21-chromosome plants of assumed male parentage have formed the basis of this report upon the offspring of *O. lata* × *O. gigas.*

The writer in August, 1907 (17) reported that the somatic cells of *O. gigas* had been found to have 28 or 29 chromosomes. In January, 1908 (10) Gates reported 28 for *O. gigas*, having determined this number from the study of reduction mitosis in the pollen mother-cells of this form. *O. lata* has been repeatedly referred to by Gates as having 14 chromosomes (7, p. 97; 8, p. 11; 10). On the first page of his paper dealing with "The Behavior of Chromosomes in *O. lata* × *O. gigas*" (13) *O. lata* is referred to as 'having usually 14 chromosomes'. He then says: "if fertilization took place in the ordinary manner, the hybrid *O. lata* × *O. gigas* would be expected to have 21 chromosomes, 7 derived from the *lata* egg and 14 (which is probably a double set of *Lamarckiana* chromosomes) derived from the male cell of *O. gigas*". If, therefore, the expectation was 21 chromosomes for hybrids of this cross, and the 20-chromosome plant derived from the package of unguarded seeds was included among the offspring of *O. lata* × *O. gigas* reported in this paper (13) there would appear to have been no reason for excluding the 21-chromosome plant of unknown male parentage. Furthermore, Gates' statement at the beginning of his paper dealing with "The Behavior of Chromosomes in *O. lata* × *O. gigas*" appears to confirm the supposition that the hybrids of this report are the 20- and 21-chromosome plants of unknown male parentage referred to in earlier contributions:

"The general results regarding chromosome numbers and distribution were obtained some time ago, and a brief statement was published (10), but the cytological evidence is here presented for the first time. Many of the drawings for these figures were completed about two years ago," etc.

The only offspring of *O. lata* × *O. gigas* which he mentions in the paper to which he refers the reader ("The Chromosomes of *Oenothera*") are the two plants of unknown male parentage which he has *assumed* to be offspring of *O. lata* × *O. gigas*. While Gates states (13, p. 180) that the 20-chromosome plant included in this report upon "The Behavior of Chromosomes in *O. lata* × *O. gigas*"[22]) was derived from a seed-package that had not been guarded, and

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22) Although in his report (13) upon the hybrid offspring of *O. lata* × *O. gigas* Gates quotes (pp. 180—181) from a report upon the Cold Spring Harbor cultures in which a variety of numbers were announced for the somatic cells of the various types of these hybrids, he states in the summary at the conclusion of his paper:

"*O. lata* × *O. gigas* has 21 chromosomes in its somatic cells, 7 of maternal origin (*O. lata*) and 14 of paternal origin (*O. gigas*). In one individual the number was 20, owing probably to the absence of one chromosome" etc.

was the individual first thought to have been *O. lata* × *O. Lamarckiana* (7), nowhere does he inform the reader that the 21-chromosome plant was derived from guarded fertilizations. No mention is made of the number of individuals grown in this culture of *O. lata* × *O. gigas*, nor does he state the number of plants in which he counted 21 chromosomes. It is not even asserted that a guarded pollination of *O. lata* × *O. gigas* had ever been made. The only information which the reader is given concerning the pedigree of the 21-chromosome plant is included in the following statement (13, p. 180):

"The plants from which these studies were made were grown at Wood's Hole, Mass., in 1905 and 1906 from seeds of de Vries."

One is left to infer from this statement that the crosses were made by de Vries, but it is possible that this is not the meaning which he intended to convey.

De Vries, in 1908, described a culture of *O. lata* × *O. gigas* as follows (6, p. 759):

„Im Sommer 1907 erzog ich 133 Pflanzen, welche schon im Juli deutlich zwei Typen zeigten. 68 Exemplare zeigten gleichzeitig die Merkmale beider Eltern, während die übrigen 65 keine Spur von *lata*-Eigenschaften verrieten, sondern genau den Bastarden von *O. Lamarckiana* × *O. gigas* glichen."

Three small cultures of *O. lata* × *O. gigas* have been grown at Cold Spring Harbor[23]), and the majority of the offspring have been found to belong to the two types described by de Vries, although, as previously reported (20), several other forms appeared in the Cold Spring Harbor cultures which were quite distinct from either of the two more common types. I devoted almost two years to the careful study of the relation of somatic chromosome number to vegetative character in the offspring of *O. lata* × *O. gigas*. During the growing season of 1908 (from the germination of the first seedling leaves early in the spring until late in October after the plants were killed by frost), attention was directed almost exclusively to the study of the vegetative characters of these and other primroses

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23) The two cultures of 1907 and 1908 were of separate parentage, but each of the two lots was derived from a single female pollinated by a single male. In referring to the hybrids of the 1908 culture reported in February, 1909 (20) Gates says (14):

"Whether these hybrids all had the same individual *O. lata* plant as mother is not stated," etc.

My mistaken usage of the term 'extracted *latas*' for first generation hybrid offspring was doubtless misleading, but he has curiously overlooked the following statement contained in the short note in which these hybrids were reported (20), and to which he refers:

"The hybrids were the progeny of a single pair of parents; the pistil parent in this case being a mutant which arose from a culture of pure-bred *Lamarckiana*, and the pollen parent a pure-bred *gigas*."

in which I was interested at the time. During the height of the season, from the first of July until the first of September, these plants were the subject of almost constant daily observation from 4, 5 and 6 A. M. until twilight. Somatic chromosome numbers have been carefully studied in 61 of these hybrids (including the first 3 grown in 1907), accurately determined in 52 of the lot, and approximately in the remaining 9.

The results obtained from the three plants of the first cross were reported (18) in August, 1907. 40 of the second cross were studied, and although, as stated in a short note[24]) given out in February, 1909 (20), the number was not exactly determined in all, the following numbers were reported to have been found among these hybrids: 15, 22, 23, "plants having *gigas* number of chromosomes (30 in each case so far definitely counted)", "and some possibly 21". 18 individuals have since been added from the second cross (some of which consisted of bud fixations only), making a total for the two cultures as stated (1907, 3; 1908, 40 + 18).

The chromosomes of the hybrids of the third culture were not examined.

Not only has the count of 21 been clearly ascertained for certain hybrids of this cross since the publication of the first note, but it has been found that this is one of the two most common numbers. However, I have never observed a 21-chromosome plant among offspring of *O. lata* × *O. gigas* having characters which 'appeared to be intermediate between *O. lata* and *O. gigas*' as stated by Gates. In each of the 16 hybrids in which I have found 21 chromosomes the characters of the pollen parent appeared to have been combined with those of *O. Lamarckiana*, commonly, and with those of *O. rubrinervis* and other forms occasionally; — various types of the pollen parent doubtless entering into these combinations. Although but 9 of the 16 came to flower (6 as annuals, 3 as biennials), the rosettes of the remaining 7 showed no sign of *lata* character that was recognized.

Also, hybrids do occur in this cross with characters inter-

24) When this note was published it was the intention to follow soon after with a detailed description of this culture; but upon attempting to prepare the report it became apparent that no logical explanation of many of the curious combinations of chromosome number and vegetative characters found in these hybrids could be offered on the basis of our almost complete ignorance of the normal behavior of *O. lata* when self-pollinated or crossed with another of its kind, and our limited knowledge of the range of variation of chromosome number among the different types of *O. gigas*. Publication has therefore been long withheld until the cultures referred to could be grown and studied. The work has now been carried sufficiently far to explain most of the points of interest among the offspring of *O. lata* × *O. gigas* and they may therefore be again referred to in this report. Full, detailed descriptions will follow in a later publication.

mediate between *O. lata* and *O. gigas*, and this type is perhaps the most common of all, but such forms invariably had 22 and never 21 chromosomes[25]). I found this to be the number for 25 offspring of *O. lata* × *O. gigas*, the majority of which appeared to combine the characters of some type of *O. gigas* with those of *O. lata*.

Lastly, hybrids do appear among the offspring of this cross having vegetative characters closely resembling those of *O. gigas*, as described by Gates for a 21-chromosome plant said to be an offspring of *O. lata* × *O. gigas*; but such hybrids have shown in each of the 8 individuals which I have studied[26]), a somatic chromosome number of not less than 28 nor more than 30 — never 21. The 9th hybrid which I found having a chromosome number in the region of that of *O. gigas* had a few characters suggestive of *O. lata*

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25) These observations do not agree with those of Geerts (16) who, as previously quoted, reported 21 chromosomes for both types of hybrids described by de Vries. The number of individuals from which Geerts made his counts is not stated in his report.

If we should find that a plant of a given type of vegetative character may have a certain number of chromosomes in Amsterdam cultures and another in those of Cold Spring Harbor, we must then no longer expect agreement in the observations of investigators from different localities, and the results of no one worker could thereafter be made of general application to the problems of mutation in the *Oenothera*. I have recently determined the chromosome number precisely in two *latas* of de Vries' culture and identification grown in 1911, and find it to be 15 in each case. It is therefore probable that the *lata* parent of the Amsterdam cultures of *O. lata* × *O. gigas* had also 15 chromosomes. Then, if the male parent had 28 chromosomes, and the processes of reduction and fertilization were normal, we should certainly expect to find 21- and 22-chromosome hybrids in approximately equal numbers among the offspring of this cross. In the Amsterdam culture of 133 plants, 68 hybrids were of one type and 65 of another. Of the Cold Spring Harbor cultures in which the chromosomes of 61 plants were studied, 25 hybrids were fouud to have 22 chromosomes, and 16 hybrids 21 chromosomes. (Other numbers also were represented, as stated above. See p. 422.) Of the 9 plants in which the chromosome number was determined only approximately, 5 had the vegetative characters of 21-chromosome plants, and therefore probably had 21 chromosomes, as they appeared to have. If such were the case, then the two cultures of this cross produced 25 hybrids with 22 chromosomes and 21 hybrids with 21 chromosomes. Considering the fact that only 61 plants were studied, this is coming pretty close to expectation. However, the chromosomes of 19 hybrids of the 1908 culture were not examined, and the vegetative characters of the majority of this number were of the 22-chromosome type. It is therefore probable that the number of hybrids having 22 chromosomes in the small Cold Spring Harbor cultures was considerably in excess of those having 21 chromosomes.

I hope to study the somatic chromosomes of the *lata-gigas* hybrid (Geerts' '*lata*-Typus') of de Vries' culture and identification in the near future.

26) Although these hybrids strongly resembled *O. gigas* in the majority of their vegetative characters, no one of them duplicated the vegetative characters of any one of the various types of *O. gigas* with which I am familiar in the same sense that the 2 *lata* hybrids of this cross duplicated the vegetative characters of *O. lata*.

combined with a larger number suggestive of *O. gigas*. It is possible, however, that these were true *gigas* characters, for several plants have since appeared among the pure cultures of *O. gigas* at Cold Spring Harbor which combined many of the common characters of *O. gigas* with others that were more suggestive of *O. lata*. However, to have found a type of *gigas* having bud-form or crinkledness of leaf (or both) resembling *O. lata* does not necessarily indicate that it owes this resemblance to *O. lata* parentage, although it does seriously complicate hybridization studies of *O. lata* × *O. gigas*[27]).

It seems probable, therefore, that the 21-chromosome plant described by Gates as offspring of *O. lata* × *O. gigas*, if such, had neither 'almost or quite the identical appearance of *O. gigas*', nor vegetative characters 'which appeared to be intermediate between those of *O. lata* and *O. gigas*', but that it was rather a 21-chromosome hybrid of the type found common among the Cold Spring Harbor offspring of *O. lata* × *O. gigas*. The strongest evidence in proof of this assumption is Gates' statement (10) that the *O. lata* × *O. gigas* hybrid 'matures an abundance of pollen'. Of the hybrids of this cross coming to flower which I have studied, only those having *gigas*-like characters in combination with a chromosome number in the region of that of *O. gigas*, — and the 21-chromosome plants excluding *lata* characters, — produced a moderate abundance of pollen. Those having *lata-gigas* characters exposed about as little pollen as *O. lata* itself.

It is unfortunate that Gates did not state whether any of the remaining 13 individuals of the supposed culture of *O. lata* × *O. Lamarckiana* (7) were also derived from seeds of the unguarded seed-package, for the paternal origin of each of the remaining members of this culture are thus left in question, including the so-called "'mosaic' hybrid".

It is now well known that *O. lata* × *O. Lamarckiana* produces the following offspring:

a) *Lata* hybrids which are in every way indistinguishable from *lata* mutants.

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27) This is but one of the numerous examples which illustrate the fact that the *Oenotheras* form a most interesting subject for mutation studies, but a much less satisfactory one (with our present knowledge) for hybridization experiments. One may pollinate yellow-budded A, for example, with red-budded B. But A may be capable of throwing red-budded mutants when self-pollinated or crossed with another of its kind, and B, under similar conditions, may throw yellow-budded mutants. Examples of such occurrences will be demonstrated in reports to follow. How is one to know, therefore, that the yellow-budded offspring have derived this character from the female parent, and the red-budded forms the sepal coloration from the male parent?

b) *Lamarckiana* hybrids which are in every way indistinguishable from *Lamarckiana* offspring of *O. Lamarckiana* × *O. Lamarckiana*.

c) Mutants.

Cultures of *O. lata* × *O. gigas* have been reported by de Vries (6), possibly by Gates (13), and by myself (20), but no *lata* offspring were stated to have appeared in either of the first two cultures, and but 2 such hybrids have been recognized in a total of 151 offspring of the above cross grown at Cold Spring Harbor in 1907, 1908 and 1909. The number of hybrids grown by Gates is not stated, — and there is considerable uncertainty as to whether he has ever grown this culture; but the total reported for the Amsterdam and Cold Spring Harbor cultures is over 284[28]). It is therefore probable that *O. lata* appears but rarely among the offspring of this cross. If the female parent of the 14-chromosome plant described in Gates' first paper (7) was known to have been *O. lata*, the presence of 4 *lata* offspring in a total of 15 would indicate that these plants originated as first stated; — by the fertilization of *O. lata* with *O. Lamarckiana* pollen. However, we are not safe in assuming that such was the case, for there are other possibilities that cannot be ignored. *O. lata* may have been fertilized by some other form which, in union with *O. lata*, may also have been capable of producing *O. lata* offspring; furthermore, one or more of these 4 plants may have arisen from the fertilization of the female parent with *O. lata* pollen. The last however, is the least probable of the three conjectures.

These assertions are based on the assumption that each of the 4 plants in question were *latas*, as stated. But Gates has reported 14 chromosomes for at least one of these plants. Was this individual wrongly identified, or was the chromosome number wrongly determined, — or may *O. lata* sometimes have 14 chromosomes?

I have determined the exact somatic chromosome number in 28 *latas*. Each was found to have 15 chromosomes, whether mutant, hybrid, offspring of mutant *lata* self-pollinated, or offspring of hybrid *lata* self-pollinated; whether grown at Amsterdam, Cold Spring Harbor, or the New York Botanical Garden; and whether derived from de Vries cultures, from plants descended from plants or seeds from de Vries, or from plants of English ancestry, in no wise related to de Vries' cultures. Since all the evidence of the Cold Spring Harbor studies points to the conclusion that all plants having the same vegetative characters throughout their life histories

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28) As previously quoted, de Vries (6) stated that he grew 133 offspring of *O. lata* × *O. gigas* in 1907. A second lot was grown in 1908 which gave the same percentages and the same characters as the first. The number of individuals composing the second culture was not stated, but it is clear that the actual number reported by de Vries for this cross is in excess of 133.

invariably have the same somatic chromosome numbers, it is probable that somatic chromosome number in *O. lata* is constant.

From the fact that an offspring of *O. lata* corresponding to the type of that described by Gates for the "'mosaic' hybrid" (7) has never been reported except as the offspring of *O. lata* × *O. Lamarckiana* and *O. lata* self-pollinated, it seems probable that this plant also originated as first stated, or as the offspring of *O. lata* pollinated by *O. lata*. However, it is possible that it may have resulted from the fertilization of *O. lata* by some other form capable (in union with *O. lata*) of producing this type.

The evidence is less clear for the remaining 8 of the 10 individuals first termed "*O. Lamarckiana* hybrid". Since this group of 10 was first described as conforming more or less completely to the characters of the pollen parent, *O. Lamarckiana*, they must, according to a well known mathematical rule, have resembled each other more or less. If, as stated in the next paper (8), the pure *O. Lamarckiana* and the *O. Lamarckiana* hybrid were 'identical in external appearance' then there can be no question but that these "*Lamarckiana* hybrids" were, with respect to each other, 'identical in external appearance'. In either case, since one of the 10 had 20 chromosomes, and possibly another of this lot, 21, it would seem probable that the remaining 8 or 9 had similar numbers and were likewise derived from seeds of the 'unguarded seed-package'. However, if I understand Dr. Gates' statements correctly, at least one of the original "*Lamarckiana* hybrids" (and probably two) was later included in a group of hybrids described as having 'almost or quite the identical appearance of *O. gigas*' (10), and still later was mentioned in a group of hybrids which 'appeared to have been intermediate between *O. lata* and *O. gigas*' (13). We have, therefore, no definite basis for speculation concerning the probable origin of these 10 plants.

While it is not probable that any of Gates' original 10 "*Lamarckiana* hybrids" (7) had the same vegetative characters as those of the "'mosaic' hybrid", it is quite as possible that one or two of the 21-chromosome plants of unknown male parentage were triploid mutants, as that they were hybrid offspring of *O. lata* × *O. gigas*.

To review briefly, a single type of 21-chromosome mutant, represented by 8 individuals, appeared among six separate cultures of *Oenotheras* at the Station for Experimental Evolution. It is possible that the "'mosaic' hybrid" of Gates' culture also belonged to this group.

A second mutant, represented by a single individual, of a type of vegetative character in some respects quite distinct from the first, was recognized in a seventh culture. The chromosome number of this plant was not determined, but was found to be not less than 20 nor more than 22.

A third mutant, also represented by a single individual, having vegetative characters quite distinct from those of the two preceeding, and having 22 chromosomes, was found in an eighth culture[29]).

All of the above 10 individuals were of known parentage and descended through guarded fertilizations from seeds or plants from de Vries.

From the foregoing it appears that the evidence is not lacking to demonstrate the occasional occurrence of triploid mutants in *Oenothera*. We may now consider the evidence at hand in regard to the relative frequency of triploid and tetraploid mutants.

Davis, in a recent publication (4), has called attention to a most important point in the following statement:

"Thus *gigas* at the most has been noted only seven times, and, since apparently the cytology of de Vries' strain alone has been studied, it is by no means certain that all of the forms reported later are the same as the first example from de Vries' cultures of 1895."

In my studies of the *Oenotheras* (as repeatedly stated heretofore) I have found no exception to the rule that all plants having the same vegetative characters from the seedling stage to the end of the flowering period, — have identical chromosome numbers. Therefore, each mutant that was certainly proven to have had the same vegetative characters as de Vries' 1895 *O. gigas* mutant, probably had the same chromosome number as the latter form. So many difficulties attend the certain identification of mutant *O. gigas* that I doubt if any one but Professor de Vries is capable of identifying it with certainty, for he alone has the mental picture of the original plant. Those of us who have gained our impressions of the vegetative characters of this form chiefly from the study of the inbred descendants may not be able to judge correctly. Furthermore, we can not rely upon the number of chromosomes for the identification of *O. gigas*, any more than we can assume that 14 chromosomes proves a plant to be *O. Lamarckiana*.

Although all of 7 individuals in question were observed by de Vries (de Vries 3, MacDougal 1, Schouten 3), the identifications were made before it was known that other forms possessed

29) Another plant was observed in a ninth culture (*O. Lamarckiana* × *O. Lamarckiana*) in 1908 which has not been included in this report as the card bearing the description of its vegetative characters was lost. However, it is marked on the 1908 chart as "*gigas*-like", and, as I have two fixations of root-tips from this plant I hope to be able to determine its somatic chromosome number at least approximately. It is probable that it also was a triploid mutant.

certain vegetative characters which strongly resembled certain characters of *O. gigas*. It is therefore not impossible that such as were identified from the rosette stage only (as for example MacDougal's "*gigas*"[30]) which was not more than two months old when observed by de Vries), may have been triploid mutants, instead of *O. gigas*. Stomps (21, p. 60) has indicated, as previously stated, that the second and third plants of de Vries' culture (5, Bd. I, p. 231) may have been such. Gates (12, p. 536) had previously suggested that there might be some question as to whether these two forms were the same as the original mutant since "neither of these plants matured".

With reference to these two mutants de Vries said (5, Bd. I, p. 231) that he succeeded in bringing the first to flower, though too late to ripen seed. He compared this individual with the descendants of the original 1895 mutant, and found that it agreed with them in all essential points. The second of the two remained a rosette and never developed a stem.

Since the first of these plants came to maturity in 1898, only three years after the recognition of the original mutant, it seems probable that there was no mistake in the identification of this form. The second of the two may have been a triploid mutant.

After triploid mutants were once definitely recognized in the Cold Spring Harbor cultures in 1908, eight were identified in 1910. This, together with the further fact that Professor de Vries believes they have appeared quite often in the Amsterdam cultures, leads me to believe that they will hereafter be found to occur with comparative frequency.

It is therefore possible that *O. gigas* may have appeared but once or twice, and that triploid mutants may occur much more frequently than tetraploid forms.

How shall we explain the fact that *O. Lamarckiana*, or a derivative of *O. Lamarckiana (O. lata)*, when self-pollinated, may produce an offspring with a chromosome number differing from that of its own?

Gates (11), in discussing the origin of chromosome number in certain forms which had been reported as having 14, 15 and possibly 16 chromosomes respectively, says:

"Disregarding the possibility that these results might be due to the well-known variation in chromosome numbers in root tips," etc.

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30) Mutations, Variations, and Relationships of the Oenotheras. D. T. MacDougal, A. M. Vail, and G. H. Shull. The Carnegie Institutions of Washington, 1907, pp. 3—4.

As previously mentioned in this report, I have determined the exact number of chromosomes in 228 plants, and have found that somatic chromosome number in *Oenothera* is constant, so far as has been observed. I have not kept a record of all the figures counted, for in some root-tips they were so abundant that this was not practical. In such cases only the best were recorded. However, an effort was made to determine the number of chromosomes in every figure that appeared at all countable, and it will be a very conservative statement to say that somatic chromosome number has been exactly determined in not less than 8000 metaphase figures, and probably many more. Exclusive of certain very rare exceptions previously referred to (and which will be fully described in later reports), the number has been found constant for the individual in all cells of the root-tips and floral tissues studied in which it was possible to determine the number. Since the majority of these studies were made from sections of root-tips, we can not explain the above phenomenon on the basis of "the well-known variation in chromosome numbers in root tips".

Although the divisions of the somatic cells may be said to be constantly regular, and as a result, somatic chromosome numbers practically invariable, we know from the researches of Gates (9, 10, 11, 13, 15), Davis (3, 4) and Geerts (16) that irregularities sometimes occur in the heterotypic mitosis of the *Oenotheras*; and from the publications of Gates (13, p. 188) and Davis (4, p. 952) that irregularities sometimes occur in the homotypic mitosis as well. We must therefore turn to the germ cells for an explanation of these mutant chromosome numbers.

The derivation of 15-chromosome mutant offspring from 14-chromosome parents can readly be explained as stated by Gates, — by one chromosome passing to the wrong pole at the reduction division of one of the two parents, and the cell having the larger number of chromosomes uniting in fertilization with a normally reduced cell. The origin of the two 15-chromosome *lata* offspring in the culture of *O. lata* × *O. gigas* can also be explained as suggested by Gates (13, 14), — by the apogamous development of these forms from female germ cells having the unreduced number of chromosomes. However, it will be shown in the following paper that there is some evidence to indicate that these two plants may have derived their chromosome numbers from the fertilization of 8-chromosome eggs by 7-chromosome sperms (or 7-chromosome eggs by 8-chromosome sperms), and that the latter may have attained their reduced numbers in the manner described by Geerts (16).

The presence of 14 chromosomes in a second generation offspring of *O. gigas* × *O. Lamarckiana* described by Geerts (16) is explained by the phenomena which he observed in the germ cells of the 21-chromosome parental form.

A number of theories have been offered to explain the origin of the tetraploid condition in *O. gigas*. One of these, namely that of de Vries and Stomps, presupposing the union of two germ cells each having the diploid number of chromosomes (and thereby allowing for the possibility of a more frequent union of a haploid and a diploid germ cell) is quite sufficient to explain the presence of the 21-chromosome mutant in cultures of *O. Lamarckiana* × *O. Lamarckiana*, and the 22-chromosome mutant in the culture of *O. lata* self-pollinated, as reported.

Since a triploid condition is represented in the 21-chromosome offspring of *O. Lamarckiana* × *O. Lamarckiana* and that which may be termed triploid in the 21- and 22-chromosome offspring of *O. lata* self-pollinated, the explanation which first suggests itself is that of the union of a reduced and an unreduced germ cell.

The question which next arises is whether this unreduced germ cell was contributed by the male or female parent.

We have the case of polyspermy in animals in which the reduced female germ cell receives a double number of chromosomes from the male, and the case of *Ascaris* in which a reduced number of male chromosomes unites with a double number from the female. From what we know of apogamy in plants, however, it seems probable that reduction fails to take place in the female germ cells more commonly than in the male. This assumption appears to be supported by the presence of 2 *lata*-like hybrids in the culture of *O. lata* × *O. gigas* and of 9 hybrids in a total of 61 for 1907 (3) and 1908 (58) having each a chromosome number of 28 (?), 29 or 30. The simplest explanation for the origin of these 11 plants is that of the apogamous development of unreduced female germ cells in the two former cases, and the fertilization of unreduced female by reduced male germ cells in the 9 remaining cases.

A total of 80 offspring of *O. lata* × *O. gigas* was grown in 1907 (3) and 1908 (77). Of this number fixations were prepared for chromosome studies from 63; chromosome numbers were studied in 61 and exactly determined in 52. These 52 offspring were distributed as follows:

Number of chromosomes	15	21	22	23	29[31])	30
Number of individuals	2	16	25	3	2	4.

31) One of the three plants of the 1907 culture was first reported (18) as having 28 or 29 chromosomes. The number has since been exactly determined and was found to be 29. Several metaphase figures of 28 chromosomes each were counted in one of the two plants marked 28 (?), but since only a few were observed, and these did not demonstrate the number as clearly as I wished, I prefer to label this plant (3368) as questionable.

The remaining 9 may be roughly classified as follows:

Number of chromosomes	21 (?)	24 (??)	28 (?)	28, 29 or 30
Number of individuals	5	1	2	1.

Of the remaining 19 hybrids in which the chromosomes were not examined, the majority appeared, from the nature of their vegetative characters, to be intermediates (as previously stated). However, several died as young rosettes and several more were dwarfed by their proximity to a large tree, so that a few remain in question, but it is certain under all circumstances that none of these had vegetative characters suggestive of low chromosome numbers.

It will be seen that we have at least 11 (which I am inclined to believe represents the full number) in a total of 80 which may have been derived from unreduced female germ cells. This is approximately one in every eight and indicates that *O. lata* at least may quite frequently produce unreduced female germ cells[32]). However, it can not be said that the 15-, 28(?)-, 29- and 30-chromosome hybrids are positive evidence of the production of unreduced female germ cells in *O. lata*, as it will be later shown that there is another explanation possible (though less probable) for the origin of each of these numbers.

It is at once apparent, however, that none of the numbers found among the offspring of this cross — 15, 21, 22, 23, 28 (?), 29 and 30 — can be explained on the basis of the union of a reduced female with an unreduced male germ cell. Therefore, while I believe that this phenomenon also occurs occasionally in the male germ cells, and that the union of a diploid female with a diploid male germ cell is the explanation of the origin of the tetraploid condition in *O. gigas*, it is probable that unreduced male germ cells are produced much less frequently than unreduced female. The true test will be the approximate determination of chromosome number in 100 or more offspring of *O. gigas* (28) × *O. lata* (15), for it may be that this phenomenon appears more commonly in the germ cells of *O. lata* than some other forms[33]).

32) This statement does not imply that apogamous offspring are frequently produced by *O. lata* or any other form. The few tests which I have made for apogamy in *O. Lamarckiana* and *O. lata* have given only negative results.

33) In this connection the somatic chromosome numbers of offspring of *O. Lamarckiana* (14) × *O. gigas* (28) and the reciprocal, *O. gigas* (28) × *O. Lamarckiana* (14) become of intense interest. In each case the union of normally reduced male and female germ cells should produce 21-chromosome offspring exclusively. De Vries grew the former culture in 1905, 1907 and 1908, and the latter in 1907 and 1908. He found (6) that, with the exception of certain linear-leafed forms appearing among the progeny of the two crosses, and a dwarf mutant in the culture of *O. Lamarckiana* × *O. gigas*, the hybrids of these two crosses were all of one type, and intermediate between the two parents with respect to vegetative

From such a cross we should expect to obtain 21- and 22-chromosome offspring in greatest number. However, should a normally reduced female germ cell unite with an unreduced male, we should have a 29-chromosome offspring resulting. Should an unreduced female unite with a normally reduced male and produce offspring, we should expect this plant to have 35 or 36 chromosomes, according to whether the combination was 28 + 7 or 28 + 8. The comparative frequency of 29-chromosome hybrids among the offspring of this cross would be some indication of the comparative

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character; — thus demonstrating that ♀ *gigas* 14 + ♂ *Lamarckiana* 7 produces the same type of offspring as ♀ *Lamarckiana* 7 + ♂ *gigas* 14.

In all cultures of these two crosses we should certainly expect to find the majority of the offspring to be intermediate, 21-chromosome hybrids. But if it is possible for *O. Lamarckiana* and *O. gigas* each to produce unreduced female germ cells occasionally, then the union of unreduced female with normally reduced male germ cells should be expected to produce 28-chromosome offspring in the culture of *O. Lamarckiana* × *O. gigas*, and 35-chromosome offspring in that of *O. gigas* × *O. Lamarckiana* (♀ 14 + ♂ 14 and ♀ 28 + ♂ 7).

While it is probable that all of the hybrids of de Vries' two cultures having intermediate vegetative characters had also intermediate chromosome numbers, we do not know, for instance, what the chromosome number of the "Zwerg-Mutante" (*O. Lamarckiana* × *O. gigas*) may have been. The linear-leafed forms in these two hybrid cultures are also of interest in this connection, since, as de Vries has said (p. 756) they appear in pure cultures of *O. gigas*. I have succeeded in counting the chromosomes approximately in but four linear-leafed *O. gigas*, and each was found to have 28(?), or more properly speaking, a number in the region of 28. Now, if the linear-leafed hybrids from de Vries' cultures of *O. gigas* × *O. Lamarckiana* and *O. Lamarckiana* × *O. gigas* were of the same type, and we should later find that such pure-bred forms and hybrids have each 28 chromosomes, it would indicate that they are produced by the apogamous development of unreduced female germ cells in the first cross, and from the fertilization of unreduced female by normally reduced male germ cells in the latter cross.

With regard to the expectations for 35-chromosome offspring *O. gigas* × *O. Lamarckiana* there is almost no evidence to indicate what these should be. Under any circumstances, since only approximately 17 or 18 plants of this cross came to flower in de Vries' cultures, we might expect all of these to be intermediates. It is furthermore possible that an individual with a somatic chromosome number as high as 35 may never be produced, although we have seen that 4 plants, having 30 chromosomes each, appeared in the 1908 culture of *O. lata* × *O. gigas*. If *O. gigas* produces unreduced female germ cells occasionally, the union of unreduced female with normally reduced male germ cells (in cultures of *O. gigas* pollinated by *O. gigas*) should produce offspring with 42 chromosomes. It seems improbable that an *Oenothera* with a chromosome number as high as this will ever be found. However, our knowledge of chromosome number in offspring of *O. gigas* pollinated by *O. gigas* is as yet very limited, and it is too early to arrive at conclusions.

Davis reported upon 12 offspring of *O. gigas* × *O. Lamarckiana* ("Notes on the Behavior of Certain Hybrids of *Oenothera* in the First Generation." American Naturalist, Vol. XLIV., Feb., 1910). 8 of the 12 were said to have been similar to *Lamarckiana* and 4 similar to *gigas* in the rosette stage. 6 of the former and 1 of the latter came to flower. These 7 plants were described as similar to one another at maturity, so it is difficult to guess what their chromosome numbers might have been.

frequency with which unreduced male germ cells are produced in *O. lata.* On the assumption that this phenomenon occurs much more rarely in the male than in the female germ cells, we could not draw conclusions from the study of 25 or 30 plants, should 29-chromosome hybrids not be found among this number of individuals.

In 1908 I covered the stigmas of a number of flowers of *O. gigas* with pollen from *O. lata.* The seed-capsules filled out nicely, and I anticipated a good harvest, but they were found in the autumn to contain only flat seed; — seed-coats without contents. The cause of this peculiar occurrence is unknown. The pollen of the *lata* used in this cross successfully fertilized its own flowers and several of *O. Lamarckiana.* Furthermore, the *gigas* of the cross produced well rounded seeds when pollinated by *O. gigas.* I may add that all my attempts to fertilize *O. gigas* with *O. nanella* and *O. Lamarckiana* in 1908 and 1909 resulted similarly. It is hoped that someone will again attempt to fertilize *O. gigas* with *O. lata.*

The union of an unreduced female germ cell with a normally reduced male germ cell will therefore explain the 21-chromosome offspring *O. Lamarckiana* × *O. Lamarckiana*, and the 22-chromosome offspring *O. lata* self-pollinated; but how shall we account for the 21-chromosome offspring of *O. lata* self-pollinated, and *O. lata* × *O. Lamarckiana*?

These can be very simply explained by the irregularities of chromosome distribution observed in male germ cells. If one chromosome occasionally passes to the wrong pole of the spindle at the reduction division, it is possible that *O. lata* may produce pollen with 6 and 9 chromosomes respectively, and *O. Lamarckiana* pollen with 6 and 8 chromosomes respectively. The union of 15- and 6-chromosome germ cells might then be expected to produce offspring with 21-chromosomes.

There is no evidence to tell us at present whether a germ cell having a reduced number of chromosomes which is less than the normally reduced number for *O. Lamarckiana* may unite in fertilization with another germ cell. But it will be shown in the following report that there is much evidence to indicate that *offspring never result from the union of two germ cells whose combined number of chromosomes is less than the diploid number for O. Lamarckiana.* It is conceivable however, that offspring might result from a combination which restored the missing chromosomes (6 + 8 or 8 + 6). However, we have no positive proof as yet that offspring are ever produced from the union of two germ cells in one of which the reduced number of chromosomes is less than the normally reduced number for *O. Lamarckiana.* Therefore, until we have more light upon the subject it will be well to consider the possibility of some other explanation for the origin of the 21-chromosome offspring of *O. lata* self-pollinated, and *O. lata* × *O. Lamarckiana*.

We know that irregularities have been found in both the heterotypic and homotypic divisions of the male germ cells of the *Oenotheras*.

We have seen that the chromosome numbers of certain offspring of *O. lata* × *O. gigas* indicate that these hybrids may have been derived from unreduced female germ cells. Furthermore, Gates (14), in testing for apogamy in *O. lata*, obtained 3 seeds from the capsules of guarded, unfertilized flowers, and Blaringhem has recently reported (1) that Miss Thomas has discovered apogamy in *O. biennis*.

On the basis of these facts and indications. Professor V. Grégoire has suggested that a further irregularity of division may be found to occur in the male and female germ cells of *Oenotheras*, and I have his very kind permission to offer this suggestion here.

Professor Grégoire's hypothesis presupposes that the majority of somatic chromosome numbers in *Oenothera* are the result of germ cell behavior as follows:

1. Irregular distribution of chromosomes as observed in the pollen mother-cells (and believed to occur also in the embryo sac mother-cell) resulting from one (or possibly more occasionally) passing to the wrong pole of the spindle, thereby producing two sets of daughter-nuclei, one having a number of chromosomes in excess of that of the normally reduced number, another having a number which is less than that of the normally reduced number. It is assumed that the former are functional, but it is possible that the latter may be so only when 7 or more chromosomes are present, — as would be the case with a 13-chromosome *O. gigas* germ cell, for example.

2. The failure of one or more chromosomes to be included within a daughter-nucleus, or the daughter nuclei at the heterotypic or homotypic mitosis, or both. It is possible that functional germ cells may result only when retaining 7 or more chromosomes.

3. The production of functional female germ cells (and possibly also male) with the unreduced number of chromosomes — resulting from the arrest of the heterotypic mitosis before the division of the nucleus has taken place.

4. Degeneration of a portion of the chromosomes, in certain forms, during the maturation process.

These conditions alone are sufficient to explain the origin of all the chromosome numbers in question. However, since many may also be otherwise explained, a fifth possibility is offered for consideration.

5. The completion of the reduction division in the normal manner, or irregularly, according to 1 or 2. The subsequent arrest of the homotypic mitosis (in the cell destined to differentiate the embryo sac) before the division of the nucleus has taken place, resulting in the production of a functional germ cell with a chromosome number double that of its reduced number.

This is assumed by Professor Grégoire to be a much less probable explanation of the origin of mutant somatic chromosome number in *Oenothera* than the irregularities of the heterotypic mitosis, and is supposed under any circumstances to be of much more rare occurrence than the latter. However, since so many of the numbers in question can be explained on this basis alone, and still more when we consider this in connection with the simple irregularities of chromosome distribution observed in the heterotypic mitosis of the male germ cells, we can not ignore it as a possibility until we have evidence to discredit it.

The works of Strasburger and others upon various species of plants have demonstrated that reduction division may be arrested at any stage of the heterotypic prophase, and even at the metaphase. Professor Grégoire believes that it is not improbable, therefore, that this phenomenon may occasionally extend even further in *Oenothera* and result in the failure of a dyadocyte to complete the homotypic mitosis.

The maturation process in *Oenothera* is, properly speaking, a continuous one from the beginning of the heterotypic prophase to the completion of the homotypic division. The figures of Davis for *O. Lamarckiana* and *O. gigas* (4, Figs. 30 and 67) clearly show that the chromosomes sometimes split as early as the middle and late anaphase of the heterotypic mitosis in preparation for the homotypic division, while Gates (7, p. 95) found that this fission sometimes appeared as early as the late metaphase of the heterotypic mitosis in the "*Lamarckiana* hybrid". There is furthermore no true resting stage between the heterotypic and homotypic divisions of this group of plants. Gates states with reference to *O. rubrinervis* (11, p. 17) that 'the nuclei never pass into the resting condition and the chromosomes never loose their identity completely, though they spread out and anastomose with each other more or less.' He also states that in *O. gigas* (12, p. 528) 'the chromosomes may stretch out and anastomose with each other', passing into what he terms a 'semi-resting condition'. Davis found that during interkinesis in *O. biennis* (3, pp. 638—639) 'the free ends of the chromosomes sometimes exhibit a tendency to branch and become united to form a loose and imperfect network', and adds "but generally most of the chromosome pairs of a nucleus are as clearly defined as in Figs. 29 and 30. . . . It is clear that the chromosomes of *biennis* maintain their individuality throughout the interkinesis," etc. The conditions which he describes for *grandiflora* (2, p. 562), *Lamarckiana* and *gigas* (4, pp. 950, 957) are much the same as for *biennis*. He states that the individuality of the chromosomes in *O. Lamarckiana* is 'generally maintained quite as clearly as is indicated in Fig. 31—33'. With reference to *gigas*, Davis said the he observed no case 'in which the chromosome boundaries were no longer distinguishable, as reported by Gates'.

In describing the interkinesis between the heterotypic and homotypic mitosis of *O. grandiflora*, Davis states as follows (2, p. 562):

"The seven split chromosomes, which were at first massed closely together, separate as the daughter-nucleus gradually increases in size, and become distributed rather symmetrically around its interior just under the nuclear membrane. A change in the form of the split chromosomes becomes then at once apparent. The ends of one chromosome of the pair swing away from the ends of the other until they lie in approximately the same plane, when the structure becomes that of two U's joined together at the bent middle regions."

Davis also describes a similar condition for *O. Lamarckiana* (4, p. 950). His figures for *O. biennis* (3, Figs. 28 and 29), for *O. Lamarckiana* (4, Fig. 31) and for *O. gigas* (4, Figs. 71 and 72) illustrate his descriptions very clearly. The chromosomes are here shown very loosely associated indeed, even entirely separated in some cases, though still lying side by side (3, Figs. 28 and 29).

If, therefore, the nucleus should fail to divide at the time when this process ordinarily takes place, and a spindle never be formed, it is quite conceivable that the tendency of these divided chromosomes to complete separation might cause the halves to become disassociated, independent units. If the reduction division preceeding had been regular, we should have a cell resulting with 14 chromosomes in *O. Lamarckiana*, and 14 or 16 in *O. lata* according to whether the 7- or 8-chromosome germ cell failed to complete the second division. In case the embryo-sac was differentiated from the 14-chromosome cell in *O. lata*, a 21-chromosome offspring could result from the union of this cell with a normally reduced male germ cell having 7 chromosomes. This would explain the origin of both the 21-chromosome mutants in question.

This phenomenon, taken in connection with possible irregularities of chromosome distribution in the male, could explain each of the chromosome numbers of the *gigas*-like offspring of *O. lata* × *O. gigas* previously referred to:

♀ 14 + ♂ 14 = 28	♀ 16 + ♂ 14 = 30
„ 14 + „ 13 = 27	„ 16 + „ 13 = 29
„ 14 + „ 15 = 29	„ 16 + „ 15 = 31.

Therefore, while it is probable that these numbers were derived from the fertilization of unreduced female germ cells, it will be well to bear the second possibility in mind.

We may now consider the combinations possible on the basis of the fore-going assumptions. In order to shorten the list somewhat, the irregularity of the second type described by Gates and Davis will not be considered. That of the type described by Geerts will also be excluded, as it has thus far been reported only for 21-chromosome hybrids. Otherwise, an attempt will be made to make the list practically complete, regardless of the fact that many of the combinations mentioned appear absurdly improbable and should never be expected to occur. It should be borne in mind that this list refers only to possibilities:

I.

1. Regularly reduced ♀ + completed apogamous development.
2. Regularly reduced ♀ + regularly reduced ♂.
3. Regularly reduced ♀ + regularly reduced ♂ failing to complete the second division.
4. Regularly reduced ♀ + irregularly reduced ♂.
5. Regularly reduced ♀ + irregularly reduced ♂ failing to complete the second division.
6. Regularly reduced ♀ + unreduced ♂.
7. Regularly reduced ♀ + unreduced ♂ failing to complete the second division.

II.

1. Regularly reduced ♀ failing to complete the second division + completed apogamous development.
2. Regularly reduced ♀ failing to complete the second division + regularly reduced ♂.
3. Regularly reduced ♀ failing to complete the second division + regularly reduced ♂ failing to complete the second division.
4. Regularly reduced ♀ failing to complete the second division + irregularly reduced ♂.
5. Regularly reduced ♀ failing to complete the second division + irregularly reduced ♂ failing to complete the second division.
6. Regularly reduced ♀ failing to complete the second division + unreduced ♂.
7. Regularly reduced ♀ failing to complete the second division + unreduced ♂ failing to complete the second division.

III.

1. Irregularly reduced ♀ + completed apogamous development.
2. Irregularly reduced ♀ + regularly reduced ♂.
3. Irregularly reduced ♀ + regularly reduced ♂ failing to complete the second division.

4. Irregularly reduced ♀ + irregularly reduced ♂.

5. Irregularly reduced ♀ + irregularly reduced ♂ failing to complete the second division.

6. Irregularly reduced ♀ + unreduced ♂.

7. Irregularly reduced ♀ + unreduced ♂ failing to complete the second division.

IV.

1. Irregularly reduced ♀ failing to complete the second division + completed apogamous development.

2. Irregularly reduced ♀ failing to complete the second division + regularly reduced ♂.

3. Irregularly reduced ♀ failing to complete the second division + regularly reduced ♂ failing to complete the second division.

4. Irregularly reduced ♀ failing to complete the second division + irregularly reduced ♂.

5. Irregularly reduced ♀ failing to complete the second dvision + irregularly reduced ♂ failing to complete the second division.

6. Irregularly reduced ♀ failing to complete the second divsion + unreduced ♂.

7. Irregularly reduced ♀ failing to complete the second division + unreduced ♂ failing to complete the second division.

V.

1. Unreduced ♀ + completed apogamous development.

2. Unreduced ♀ + regularly reduced ♂.

3. Unreduced ♀ + regularly reduced ♂ failing to complete the second division.

4. Unreduced ♀ + irregularly reduced ♂.

5. Unreduced ♀ + irregularly reduced ♂ failing to complete the second division.

6. Unreduced ♀ + unreduced ♂.

7. Unreduced ♀ + unreduced ♂ failing to complete second division.

VI.

1. Unreduced ♀ failing to complete the second division + completed apogamous development.

2. Unreduced ♀ failing to complete the second division + regularly reduced ♂.

3. Unreduced ♀ failing to complete the second division + regularly reduced ♂ failing to complete the second division.

4. Unreduced ♀ failing to complete the second division + irregularly reduced ♂.

5. Unreduced ♀ failing to complete the second division + irregularly reduced ♂ failing to complete the second division.

6. Unreduced ♀ failing to complete the second division + unreduced ♂.

7. Unreduced ♀ failing to complete the second division + unreduced ♂ failing to complete the second division.

It will be apparent to the reader from the fore-going that there is little left to worry about unless he is in search of a number that will disprove the theory that somatic chromosome numbers owe their origin to the behavior of the germ cells.

It is not necessary to attempt an estimate of the relative frequency with which these various combinations should be expected to occur. It would be an impossible undertaking. But it will be at once apparent that by far the greater number should come under the head of I. 2; after that a very much smaller number should appear under I. 4 and III. 2, and perhaps a still smaller number under V. 2 or III. 4. Further than that I will not attempt to guess. Many, of course, are far beyond the limits of probability.

While combinations with germ cells failing to complete the second division have been included in this list, it is hoped that it will be recalled that very little emphasis is laid upon these. They are mentioned merely because of the fact that it is possible to explain certain somatic chromosome numbers on the basis of the assumption that the germ cells sometimes fail to complete the homotypic division.

If, in addition to the above, we consider the possibility of one or more chromosomes occasionally failing to be included within the daughter nucleus or daughter nuclei at the telophase of the heterotypic or homotypic mitosis, the list of possibilities for somatic chromosome numbers could be much extended. A further complication would be added should we later find that chromosome degeneration may occasionally occur in any form having a number of chromosomes in excess of 14. Evidence will be brought out in the next paper to show that there is some indication of this in both *lata* and *gigas.*

Gates, in July, 1907 (8, p. 13), seeking an explanation for the chromosome conditions which he had reported for what was then supposed to be offspring of *O. lata* × *O. Lamarckiana* (*lata*, 14; "*Lamarckiana* hybrid", 20 or 21) offers among a number of others the following suggestions:

"(2) It is possible that *O. lata* might produce two kinds of eggs, having respectively seven and fourteen chromosomes. If both these kinds of eggs were fertilized with *O. Lamarckiana* pollen and produced embryos, we should have plants resulting with fourteen and twenty-one chromosomes. The difficulty here, however, is that on such an hypothesis the union of seven *lata* chromosomes would produce a *lata* plant; while the union of fourteen *lata* chromosomes with only seven *Lamarckiana* chromosomes would produce a *Lamarckiana* plant, a situation which is highly improbable, to say the least.

"(3) Another possibility is that all the eggs of *O. lata* have the unreduced number of chromosomes, and that part of them develop without fertilization (parthenogenetically), producing *O. lata* plants with 14 chromosomes; while others are fertilized with *O. Lamarckiana* pollen and produce *Lamarckiana* plants having twenty-one chromosomes (fig. 3). This assumption in perhaps as reasonable as any, but no case is known of an unreduced egg being fertilized."

We now know that *O. lata* has 15 chromosomes and *O. Lamarckiana* 14. It is furthermore impossible to concede that all the eggs of *O. lata* may have the unreduced number of chromosomes, as Gates has here suggested. If such were the case, and *O. lata* had 14 chromosomes as he has stated, the fertilization of *O. lata* by *O. lata* should give only 21-chromosome offspring, provided the maturation process in the male and female germ cells had been regular. If *O. lata* has 15 chromosomes as I have found, then the fertilization of unreduced female germ cells with normally reduced male, should give 22- and 23-chromosome offspring only.

I have determined the somatic chromosome number of but 33 offspring of *O. lata* self-pollinated thus far (exclusive of a few plants of Dr. D. T. MacDougal's culture, not derived from seeds or plants from de Vries), and but one of this number was found to have 21 chromosomes and one other 22. No one of the remaining 31 had a chromosome number anywhere in the region of 21, 22 or 23.

Later (13), in referring to the somatic chromosome numbers reported for the Cold Spring Harbor culture of *O. lata* $\times$ *O. gigas*, Gates states that the two *lata*-like hybrids of this cross having each 15 chromosomes may have been apogamously derived. In a short paper published a few months later (14) he again refers to the subject and makes the following statement:

"Whether these hybrids all had the same individual *O. lata* plant as mother is not stated, but if this was the case and the mother had fifteen chromosomes, then we might expect the two *lata* plants in the offspring both to have fifteen chromosomes, and the hybrids of class III to have twenty-one or twenty-two chromosomes ($14 + 7$ or $14 + 8$), while in the case of the *O. gigas*-like plants which are stated to have had thirty chromosomes in the individuals in which a count was made, the expectation would perhaps be twenty-nine ($15 + 14$).

"How the *O. gigas*-like individuals having about thirty chromosomes originated must, however, be a matter of conjecture at the present time."

While it is therefore impossible that all of the female germ cells of *O. lata* may be produced with the unreduced number of chromosomes, we have seen that the evidence is more in conformity with the first of the two suggestions offered by Gates, namely, the production of two kinds of eggs having respectively the reduced and unreduced number of chromosomes.

However, in a paper published in 1909 (12) and another published in 1911 (15) — as quoted at the beginning of this report — he discredited the probability of an unreduced germ cell uniting with a reduced germ cell on the basis of the assertion (15) that 'we should have a mutant occurring with twenty-one chromosomes and that 'such a mutant has never yet been found, and all the other mutants which are known have fourteen chromosomes, as in *O. Lamarckiana*'.

In his first published report upon germ cell studies in the *Oenothera* Gates makes the following statement (7, p. 108):

"The difference found in the number of chromosomes in the mutants of Oenothera very strongly favors considering these forms of "specific" rank. I think it will be evident to anyone studying carefully and comparing the different mutants, that they are quite as distinct and easily distinguishable as are the species of any ordinary genus. The differences in the number of chromosomes is still further and, I think, conclusive evidence that the forms are distinct "species".

So far as I have been able to discover, no mention has been made of differences of chromosome number in mutants of *Oenothera* previous to Gates' first paper. In this contribution he mentions no mutant with a chromosome number differing from that of *O. Lamarckiana.* Even *O. lata* of this culture he repeatedly states has 14 chromosomes. It has been previously pointed out that one or more of the 15 plants to which he refers in this report may have been new mutant forms, but he did not so consider them, since 4 were said to have been *O. lata*, one a "'mosaic' hybrid" and 10 "*O. Lamarckiana* hybrids".

The first mention of a mutant with a chromosome number differing from that of *O. Lamarckiana* was published by the writer 6 months later (17), when it was stated that *O. gigas* (not a mutant, but a direct descendant of de Vries 1895 *O. gigas* mutant) had been found to have 28 or 29 chromosomes. I have therefore been unable to discover the authority for this statement concerning the 'difference found in the number of chromosomes in mutants of Oenothera'.

In a paper published in 1909 (12) Gates asserts in referring to *Oenothera* mutants that 'the chromosome number of nearly all is now known', and finally, in a very recent paper (15), speaking of the chromosome number in *O. gigas* says (as previously quoted) "all the other mutants which are known have 14 chromosomes as in *O. Lamarckiana*."

In the next report I hope to outline the results obtained from the study of somatic chromosomes in 300 *Oenotheras*. It is realized that this number is very small, and it is not expected that all the theories discussed in this report will stand or fall on the basis of these limited observations; however, they are sufficient to demonstrate the fact that many mutant derivatives of *O. Lamarckiana* have a chromosome number differing from that of the parent. I may anticipate a future report sufficiently to state that I have found many quite distinct types of mutants with 15 chromosomes, and some even with 16. There is also considerable evidence to indicate that the former are produced as frequently by *O. Lamarckiana* as 14-chromosome mutants, and perhaps even more commonly.

Somatic chromosome number in the *Oenotheras* can usually be determined approximately in any form (from sections of root-tips) in comparatively short time, but the exact determination of number beyond all chance of error is generally a long and tedious process, and such studies proceed but slowly. It is therefore hoped that more workers will come into the field, and that especial attention will first be given to the accurate determination of somatic chromosome number (from sections of root-tips) in the various mutants of *O. Lamarckiana* and other forms. Scarcely a start has yet been made in this direction.

Other subjects of present interest may be mentioned:

1. Verification of the count of 15 chromosomes for *O. lata*.

2. The determination of somatic chromosome number in the various types of *O. gigas*.

3. Verification of the count of 22 chromosomes for the offspring of *O. lata* × *O. gigas* which appears to combine the characters of the two parents. It is very important that the chromosome number of each parent shall have first been determined.

4. The recognition of triploid mutants, and the exact determination of somatic chromosome number in all such plants; a careful study of the reduction process in the first and last flowers of 21-chromosome mutants and hybrids. Early and late flowers from the same regions of the plant should then be self-pollinated, and the position of each capsule carefully recorded. Sowings from each capsule should be made separately. The next step is the exact determination of somatic chromosome number in as many offspring as possible.

5. An approximate estimate of the number of somatic chromosomes in many offspring of *O. Lamarckiana* × *O. gigas* (after

having first ascertained the number in each parent) to determine whether 14- and 28-chromosome offspring are occasionally produced. Also in *O. gigas* × *O. Lamarckiana* to determine whether 42-chromosome offspring may occasionally result from this cross.

6. Extensive tests for apogamy in *Lamarckiana*, *lata*, *gigas* etc.

In order to obtain a large number of figures in metaphase (which is the only satisfactory stage for the exact determination of somatic chromosome number), root-tip fixation should be prepared between the hours of 10 A.M. and 12 : 30 P.M. It is desirable to make seven or eight fixations from plants with a large number of chromosomes.

It will be of great benefit to the reader if all workers reporting upon cell studies of the *Oenotheras* in the future will clearly state, not only the number of individuals in which chromosome counts have been approximately determined, but the number in with these counts have been certainly ascertained. Definite statements as to whether the plants have been derived from open or guarded pollinations, together with some simple and precise description of the vegetative characters of the plants in question, will be of great assistance to other workers.

It has been my great privilege to prepare this report in consultation with Professor V. Grégoire, and I am most deeply indebted to him for many suggested interpretations and lines of thought leading ultimately to the demonstration of the harmonious relationship existing between practically all of the observed phenomena thus far reported for the germ and somatic cells of *Oenothera*.

April 9. 1912.

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Explanation of Figures.

The two figures of chromosomes were drawn with the aid of a camera lucida. The lenses used were the Zeiss compensating ocular No. 8 and the 2 mm. immersion objective (aper. 1.30).

Fig. 1. Plant No. 5558, mutant of *O. Lamarckiana* × *O. Lamarckiana*. Figure in metaphase from section of floral tissue, showing 21 chromosomes.

Fig. 2. Plant No 4453, mutant of *O. lata* self-pollinated. Figure in metaphase from a section of a root-tip showing 22 chromosomes.

Fig. 3. Plant No. 5420, 21-chromosome mutant of *O. lata* self-pollinated.

Fig. 4. Plant No. 5589, mutant of *O. Lamarckiana* × *O. Lamarckiana*, having not less than 20 nor more than 22 chromosomes.

Fig. 5. Plant No. 4453, 22-chromosome mutant of *O. lata* self-pollinated.

Fig. 6. Plant No. 5958. *O. Lamarckiana* offspring of *O. Lamarckiana* self-pollinated. Typical appearance at the close of the flowering season.

Fig. 7. Plant No. 4673. *O. gigas* offspring of *O. gigas* self-pollinated.

Zur Chromidienfrage und Kerndualismushypothese.

Von B. Swarczewsky (Kiew).

I. Über die „generativen" Chromidien bei den Gregarinen.

Eine Bildung von generativen Chromidien mit nachfolgender Rekonstruktion „sekundärer" Kerne aus denselben, welche sich nach zweimaliger mitotischer Teilung zu Kernen der Gameten verwandeln, ist bis jetzt nur bei *Gregarina cuneata* (Kuschakewitsch, 1907) und bei *Lankesteria* sp. (Swarczewsky, 1910) beobachtet worden. In allen den vielen anderen Fällen lässt sich die Bildung der geschlechtlichen Kerne auf eine vielfache aufeinanderfolgende Teilung des primären Kernes der enzystierten Gregarinen zurückführen.