Tetraploid Mutants and Chromosome Mechanisms.

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(Schluss.)

In two subsequent papers (1911b and 1912) Artom has contributed further studies on the sexual (2 ✕) and parthenogenetic (4 ✕) races of Artemia. He has obtained the parthenogenetic variety from several Italian localities and also from Odessa, while the sexual variety is known from Cagliari and also from Salt Lake, Utah. In the last paper the size-relationships of the Cagliari and Capodistria races are further investigated, and a few of the results may be mentioned. The method used was to measure the area of the drawings of nuclei, treating them as ellipses. In this way the "nuclear area" in the two races was found to be directly proportional to the quantity of chromatin — a confirmation of Boveri's law. It is unfortunate that the volume of the nuclei was not determined, so that the results could be directly compared with the law obtained by Gates, Tischler, and others in plant-cells and nuclei.

The "nuclear area" of the cells of the intestine in the median abdominal region of the metanauplius of the two races was found to be as 1 : 3.7. The cells from the thoracic part of the adult

intestine in the two races gave the result 1 : 2.7, while for the abdominal region of the intestine it was 1 : 3.5, and for the optic ganglia 1 : 2.2.

The average length of the metanauplius of univalens was 0.53 mm, and of bivalens 0.70 mm. The relative length of the larvae of the two races in four different stages was also found to be as 1 : 1.3, and these differences in size are said to be equivalent to the differences in the salinity of the water in which they live.

Artom concludes that the quantity of chromatin in the cells of bivalens is more than double that in univalens, but the "area" of the nuclei and cells is directly proportional to the quantity of chromatin; the number of cells being approximately the same in both.

In an interesting paper which was overlooked, C. Müller (1909) has described the chromosomes of three species of Yucca; *Y. guatemalensis* Back., *Y. aloifolia* L. and *Y. Draconis* Torr. In all three species there are five pairs of large chromosomes, varying in length, and 44—46 small, globular ones, making a total of 54 or 56.

In a later paper (1912) Müller has studied the chromosomes in a large number of genera of Liliaceae and Amaryllidaceae. The numbers run from 10—12 (2 *X*) in four genera, to 14 in *Aloe Hanburyana*, 16 in *Galtonia candicans* and *Hyacinthus orientalis*, 16—18 in *Haemanthus*, 18 in Chionodoxa, 20 in Amaryllis, Brunswigia, Veltheimia and (?) Scilla, 22 in Nerine, 26 in *Bulbine annua*, 30—32 in *Eucomis bicolor*, 32—34 in *Listera ovata* (cf. 32, Rosenberg, 1905), 36—38 in Muscari, 54 in *Albuca fastigiata*, 56 (?) in Yucca, and about 60 in *Beschornia superba*. Accurate measurements of many of these chromosomes are given. From these results it is evident that the chromosomes of the two families are, or have been, in a state of flux, and it is fairly obvious that the high chromosome-numbers came from the transverse segmentation of certain pairs. These formed a varying number of short chromosomes, while the remaining pairs retained their original condition. Studies of this group have not yet been sufficiently extensive to determine whether possibly the number of these small chromosomes varies in different individuals, but it is possible that a condition might be disclosed resembling that of the supernumerary chromosomes described by Wilson in Metapodius and other Hemipteran genera. This group of plants ought to be a very favourable one for studies of the chromosome behaviour in hybrids.

Wilson (1909) has listed the chromosome numbers known in the insects up to the date of his paper, with the following results: In the family Pentatomidae the somatic number in different species is known to range from 10 to 14, 16 and 26; the Pyrrochoridae the female somatic number ranges from 12 to 14 and 24; in the Coreidae

from 14 to 16, 22, 24, and 26; in the Chrysomelidae from 22 to 28 and 30, while Wieman (1910) has found 36 in *Leptinotarsa signaticollis*; Tenebrionidae, female somatic number, 20; Elateridae, 20; Jassidae, 24; Blattidae, 24; Fulgoridae, 28; Aeschinidae, 28; Galgulidae, 38; Diptera, 12. Many others have been added since. The researches of Wilson and others have made it highly probable that various types of phylogenetic change in the chromosome number occur in the insects, and it is not improbable that this includes tetraploidy in some cases.

In the light of the facts, above cited, that Strasburger, as well as the Marchals and others, have given their adherence to my views, it is surprising to find de Vries stating in a recent paper (1912, p. 34), „Zwar hat Gates eine abweichende Ansicht aufgestellt und behauptet, dass die Verdoppelung erst nach der Befruchtung geschehen sein sollte." De Vries apparently thinks it vital to his theory of mutation, that all the mutants should originate from changes occurring during sporogenesis, but it is by no means clear why this should be the case. It seems reasonable to suppose that changes in the germ plasm may also occur at other points in the life cycle. The moss mutation above-mentioned is a case in point. Also numerous cases of bud mutations are known, and I shall describe a new one in the present paper. I have already shown as clearly as it can be shown in breeding experiments, that the mutant *O. rubricalyx* originated as a hybrid between a mutated (*rubricalyx*) germ cell, and a normal germ cell of the parent *rubrinervis*. But it by no means follows that mutational changes in *Oenothera* are confined to this part of the life cycle[12].

Stomps has recently observed triploid (3 *X*) mutants, a) in a single individual from *O. Lamarckiana* (1912 a, p. 413) having 21 chromosomes, and characters intermediate between the parent form and *O. gigas*. For such mutants he suggests the name *semigigas*. b) Eleven Hero or 3 *X* mutants were observed from crosses between *Lamarckiana*, *rubrinervis* or *lata* on the one side, and *cruciata*, *muricata*, *biennis* Chicago and *Millersi* on the other side. These 3 *X* forms are easily distinguishable by being deep green and of larger size. They were found by de Vries to occur in about three in a thousand.

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12) In a former paper (Gates, 1910) I described what appeared to be a sectorial chimera of *Oenothera*, derived from seed, and during the past season I studied a still more striking case of a periclinal chimera occurring in a race of *O. Lamarckiana* from seeds collected in 1911 at St. Anne's, Lancashire. The latter individual had its leaves edged with white, owing to the absence of chloroplasts from the hypodermal layer. The interest of these cases lies in the fact that they came from seeds, and the original change is certainly to be sought in the young embryo. There seems no reason why these should not be classed as vegetative mutants of a certain type. The cultivated varieties of holly, pelargonium and other plants with white leafmargins must have had a similar origin.

In another paper (1912 b, p. 533) Stomps describes (c) a single mutant from *O. biennis* having 21 chromosomes, which he calls *O. biennis semigigas* [13]).

Miss Lutz (1912) has also found 5 triploid mutants from *Lamarckiana* and 3 from *lata* $\times$ *Lamarckiana*, having 21 chromosomes in their root tips.

Both Stomps and Miss Lutz apparently assume that this proves tetraploid mutations to have originated through the union of two diploid (2 *X*) germ cells and by this method only. Their conclusion in no way follows, however, for if all the pollen grains are haploid (*X*) as in other plants, or if the diploid pollen grains fail to function, the origin of both triploid and tetraploid mutants can still be easily explained; namely, that the former originate from the fertilization of a diploid egg by a haploid male call, the latter from the apogamous development of a tetraploid megaspore mother cell, such as was observed by Geerts. That a triploid mutant can also be produced by the union of a diploid male cell with a haploid egg, will only be proven when it is shown that diploid pollen grains occur and are functional. At present there is no certain evidence for this, while there is direct evidence for the occurrence of tetraploid megaspores in the above-cited observation of de Vries' pupil, Geerts. This also, despite the fact that studies of pollen development in the Oenotheras have been much more numerous than studies of megaspore development, and notwithstanding the further fact that the number of microspore tetrads observed has been thousands of times greater than the number of megaspore tetrads.

Races of *O. gigas*.

In the paper already cited (Gates, 1909a, p. 536) I mentioned that the *gigas* of de Vries' cultures is all descended from one individual, but that two other mutants more or less resembling *gigas* were observed. I suggested that the latter perhaps represented a different form, and it now appears that they may have been triploid in character. Two independent cases of the appearance of *gigas* forms have since occurred, and these I shall now describe. The first I received in 1909 from the Botanical Garden at Palermo, Italy, under the garden name *O. cognata*. My first culture from these seeds, grown at the Missouri Botanical Garden, is described in a paper now in press in the Transactions of the Linnean

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13) During the past summer I also (Gates, 1912 a) discovered mutants in a cultivated race of *O. biennis*, including *O. biennis lata*, *O. biennis laevifolia* and *O. biennis rubrinervis*. These corresponded to, though not agreeing with, the *Lamarckiana* forms, and were called parallel mutations.

Society [14]). This culture showed that the plants in their adult rosette stage were identical with the *gigas* of de Vries, though they passed through an earlier rosette stage which was quite unlike *gigas* or any other type. This year (1912) I grew at the John Innes Horticultural Institution twelve plants from the remaining original seeds [15]). These were all, with certain exceptions to be mentioned later, identical with the *gigas* of de Vries in all stages of their development. The 1909 plants had all remained rosettes, so I obtained no seeds from them, and I attribute the peculiar early rosette development to the very hot, humid summer climate of St. Louis.

Subsequent inquiry from the Director of the Palermo Garden elicited the information that the race had originated there several years previously (apparently from a single plant) but that they had subsequently lost the strain. The seeds from my culture of this year are therefore probably the only ones of this strain in existence.

The accompanying photographs (figs. 1 and 2) [16]) show the rosette and adult stages of typical individuals in my culture of 1912. The pedigree numbers of certain individuals will be given for the sake of convenience in future references. A preliminary examination of the chromosomes in typical plants shows their number to be 28. I shall refer to this race as *O. gigas* Italy.

Every individual came into bloom in the English climate, the seeds having been sown in January and the young rosettes planted out in May. Nine of the adult plants were uniform and typical, though showing certain very slight differences among themselves in one or two points. Of the remainder, No. I, 9 corresponded probably, to *gigas oblonga*, having a less stout stem, smaller leaves which were nearly free from crinkling and possessed a nearly even margin and a shape similar to *oblonga*. No. I, 12, when fully developed, differed from the typical plants to a less degree, having somewhat smaller leaves which were more deeply crinkled, and more red on the buds (colour pattern 3—5, see Gates, 1911 b, pl. 6). The rosette, however, was typical (fig. 1). The last aberrant plant, No. I, 4, differed from the type only in being distinctly smaller in all its parts, though not small enough to be called a dwarf. Its flowers were rather smaller than in *O. Lamarckiana*. This was one of the most interesting plants in the culture and will be referred to again, later. Its leaves and flowers (see table II),

though smaller, were otherwise like the type. The following descrip-
tion of the typical adult plants is taken from my notes.

Description: Stem leaves very large, thick, broad, crinkled,
prominently repand-denticulate, midribs white. Stem very stout,
not quite so tall as neighbouring *Lamarckiana* plants, but later in
developing. Central stem surrounded by a circle of basal suberect
branches arising from the rosette[17]). Later, short lateral branches

Fig. 1.   *O. gigas,* Italy.   Rosette July 8.   Cult. $^{227}/_{12}$ I. 12.

begin to develop from the central stem. Buds very large, early
ones quite green or yellowish, later ones yellowish with pale red
colour pattern (1—5, see above). The petals, and to a less extent
the sepals, are exceptionally long, so that the stigma is enclosed
by both, and does not project beyond the petals in the bud as
frequently happens in other forms.

The following table (II), made from the measurements of four buds
from typical plants and several from the smaller plant (Nr. I, 4),

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17) This is erroneously stated by Miss Lutz (1912, p. 392) to be distinctive
of *gigas,* but precisely the same condition occurs not infrequently in *Lamarckiana.*

Table II[18].

Comparative measurements of flowers in *O. gigas* races and in *O. Lanarkiana*.

	Length of bud cone	Diameter of cone at base	Length of sepal tips	Length of hypanthium	Length of ovary	Thickness of ovary	Thickness of hypanthium	Dimensions of petals	
								Length	Breadth
O. gigas Italy. type (227/12). (four flowers)	40—45 mm	13.75—15.5 mm	5.5—8 mm	40—43 mm	13.5—14 mm	about 6×5 mm[19]	about 4.5×5 mm[19]	50 mm	64 mm
227/12 I. 4 (smaller)	29—34 „	10—12 „	6—8 „	29—35 „	11—13 „	4.5 „	nearly 4 mm	45 „	60 „
O. gigas Sweden, type (213/12) . . .	52 „	13.5 „	11 „	45 „	18 „	5.5×5 „	4.5 „	59 „	75 „
Small-flowered branch on 233/12 I. 4	34 „	11 „	—	29 „	10 „	4 „	3.5 „	—	—
O. Lanarckiana two typical flower	35—39 „	9—9.5 „	8—6.5 „	30—36 „	10—11 „	3.5 „	3 „	—	—

18) These measurements are nearly all smaller than those of Nilsson (1912, p. 134), but his flower measurements were made earlier in the season than mine. The measurements he gives for buds are apparently (length of cone + sepal tips) × (thickness of cone at base), though this is not specifically stated.

19) The ovary and hypanthium are not terete, as in other forms, but usually distinctly flattened.

shows the comparative dimensions of the flowers. To this are
added measurements of two typical flowers from *O. gigas* Sweden,
to be described shortly.

One of the typical plants above described (No. I, 10) showed
an interesting peculiarity in the absence of an absciss layer between

Fig. 2.   *O. gigas* Italy, cult. $^{227}/_{12}$.   I. 5.

the hypanthium and ovary, so that all the faded flowers remained
permanently attached to the plant, even weeks after blooming.
Plant No. I, 5, showed the same feature partly developed (cf. fig. 2)
and it appeared slightly in No. I, 11. It seems that in all the
giant races of *Oenothera* the flowers tend to remain attached longer
than in other forms.

The next race to be mentioned I shall refer to as *O. gigas*, Sweden. It originated from a single mutant which appeared in 1907 in a pedigree culture of about thirty *O. Lamarckiana* plants which were grown by H. Heribert Nilsson (1909) at Lund. My seeds of this race were kindly sent to me by Nilsson, for com-

Fig. 3. *O. gigas* Sweden. Rosette July 10. Cult. $^{233}/_{12}$. II. 3.

parison with the other races of *O. gigas*. They correspond to the "Komb. 7" of his paper. Though clearly a giant form, the following description will show that it is a characteristic race, differing constantly from the *gigas* from Amsterdam and the one from Palermo [20]. From Nilsson's pedigree seeds I grew thirty-six plants as culture $^{233}/_{12}$. They were a uniform progeny except for certain

20) Since this was written, an important paper by Nilsson (1912) has appeared, with descriptions of this and other forms based upon extensive cultures. Such studies as these are particularly valuable in making possible a correlation of the results obtained by various investigators. The facts stated in my paper with regard to *O. gigas* Sweden are to be regarded as merely confirmatory of the previous studies of Nilsson with that interesting mutant. The value of Nilsson's experiments in greatly enhanced by the fact that his cultures were derived from an independant, and slightly different, race of *O. Lamarckiana* from a garden in Southern Sweden.

individuals to be mentioned later. Fig. 3[21]) shows a typical mature rosette photographed July 10th.

Until the end of June the rosettes were indistinguishable from culture $^{227}/_{12}$. Then the rosettes were found to be larger and with more jagged teeth at the base of the blade in the older leaves.

Fig. 4.   *O. gigas* Sweden, cult. $^{233}/_{12}$.

When fully developed, as in fig. 4[22]), they were found to differ constantly from culture $^{227}/_{12}$ in the following characters.   1) The stem leaves have conspicuous red midribs, and the midribs and petioles were also pink on their ventral surface.   2) The stem leaves are longer and

21) Cf. Nilsson (1912, textfig. 30, p. 173).
22) Cf. Nilsson (1912, textfig. 27, right, p. 165).

less crinkled, being often nearly smooth (cf. fig. 4). 3) The margin of the stem leaves is much more conspicuously repand-dentate, often with jagged teeth near the base (see fig. 5). 4) In habit (cf. figs. 2 and 4), having the basal branches more spreading, and more numerous and longer stem-branches, which developed secondary

Fig. 5.   Upper row, three stem leaves from *O. gigas*, Sweden.
Lower row, three stem leaves from *O. gigas*, Italy.

branches later in the season. The plants were thus distinctly larger and more bushy than *O. gigas*, Italy. 5) The flowers were decidedly larger, as shown in Table I. Long hairs on the bud cone were also more numerous and from larger papillae, the hairs themselves being also longer. 6) An interesting constant difference was found in the capsules[23]), which in *gigas* Italy were contracted at the extreme base, as in most of the related forms, while in *gigas* Sweden, they were usually expanded vertically to give a broad base of attachment reaching as much as 14.5 mm in one dimension. The capsules are also longer[24]) than in the former, which is doubt-

23) Nilsson (1912), in his recent paper gives (p. 133) a fuller account of the contrasting characters between these two giant types, but apparently has not observed this difference in the fruits.

24) The fact that this race is decidedly larger in stature, including size of stems, leaves, flowers, ovaries and capsules, may not be without significance. I

less associated with greater seed-production (see table III). 7) They were slightly slower in development, and five of them remained rosettes. 8) As shown later, the flowers produced considerably more pollen than *gigas* Italy, though not so much as *Lamarckiana*.

Fig. 6.   *O. gigas* de Vries, Amsterdam.   Narrow-leaved Rosette.   July 10.
Cult. $^{180}/_{12}$.

There is thus decidedly less sterility, both in anthers and ovaries, than is the case with *gigas* Italy.

The individuals of this culture showed a certain amount of variation in crinkling (though in most the leaves were smooth or nearly so), but No. I, 5 had constantly narrow leaves though differing from the type in no other respect. Another (II, 28) was very distinct. It was late in developing but had broad leaves very much crinkled with less toothed margins, rather closely resembling the typical *gigas* of de Vries[25]).

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understand that the chromosomes are being studied by Nilsson. By analogy I should anticipate that the race will have at least 28, and possibly 30 or more chromosomes

25) Nilsson describes and figures this form (1912, p. 166, textfig. 27 left). He attributes the differences between this individual and the type to the absence of the factor for red leaf-midribs, which brings about correlatively all the other changes in leaf and habit. It would be interesting to know how the numbers of chromosomes in these two forms compare.

A more interesting departure, which must, I think, be classed as a bud mutation, was observed in No. I, 4. This plant was typical of the culture, but bore basal side branches having decidedly smaller flowers and leaves (cf. table II), the former being about

Fig. 7. *O. gigas* de Vries, Amsterdam. Cult. $^{150}/_{12}$. Same plant as fig. 6, Aug. 26.

the size of those in *Lamarckiana*. It seems reasonable to consider this a mutative bud reversion, though I have not yet counted the chromosomes.

A third culture of *gigas* which I grew during the last season, was derived from pure seeds received from de Vries several years ago. Only one germinated this year, and it proved to be a very

narrow-leaved form [26]). Fig. 6 shows the rosette photographed July 10th, when the central shoot was beginning to develope, and fig. 7 shows the mature plant. This is the first time I have succeeded in getting one of the very narrow-leaved variants to flower, but it showed a very high degree of sterility, producing no good pollen. Its chromosome number will be determined later. The buds were much smaller than in normal *gigas*, but were not measured. They were greenish in colour, and the earlier ones showed asymmetry in shape similar to that of *lata*. The petals were also somewhat crumpled, and the sepals rather short. The stamens were sinuous and contained but little pollen, one stamen being found attached throughout its length to a petal.

Table III.
Measurements of typical capsules.

	Length of capsule	Width of capsule just above base
O. gigas Italy	27—28 mm	9.5—10.5 mm
O. gigas Sweden	36—39 „	8—8.5 „
O. Lamarckiana	30—34 „	7.5 „
O. lata	19—24 „	7.5 „

The above Table gives the measurements of several capsules from each of the races mentioned. The *lata* capsules are shortest, because they produce fewest seeds. In *O. gigas* Italy the capsules are strongly rhomboidal in cross-section, their greatest diameter being, as in most of the other forms, just above the base. Here again the short length of the mature capsules is undoubtedly to be attributed to the small number of ovules which it matures. As shown in Table II, the ovary before fertilization is longer than in *O. Lamarckiana*, while in *O. gigas* Sweden it is very much longer, as is also the ripe capsule. The fact that the capsule of the latter usually has a broad expanded base has already been referred to.

The Pollen Grains.

During the past season I devoted some time to the examination of *Oenothera* pollen, the various types of grains being determined numerically, with interesting results. The pollen of *gigas* is charac-

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26) I have repeatedly found that when old seeds are sown and only one or two germinate, the plants produced are almost invariably aberrant. This would seem to indicate that the seeds which produce aberrant individuals are more viable. I have had this experience too frequently to attribute it to mere chance.

teristically 4-lobed, though grains with five and more lobes occur, while that of the other forms is usually 3-lobed[27]).

The method used was to mount as much as possible of the pollen from one anther at a time in a drop of water, and then examine it and count the number of grains of the various types. Of course, only a fraction of the whole number of grains in an anther can be observed and recorded in this way, but by examining all the pollen grains sufficiently isolated in a given field of the microscope and then passing on to another field and examining all in sight, one may be quite sure of obtaining the correct proportions of the various types. In determining "good" and "bad" grains, those were considered "bad" which showed any signs of shrivelling or distortion, but probably the real proportion of non-viable grains would be somewhat larger. When carried on in this way, the examination of the pollen from a single flower requires a considerable time. Nevertheless, it is much more expeditious than cytological methods, and I believe it may prove a useful auxiliary method in determining the nature of the different types of pollen grains.

It has apparently not occurred to Miss Lutz that the quadrangular and triangular types of pollen grain may contain respectively 2 X and X chromosomes, or thereabouts. If this were the case, a critical statistical examination of the pollen in the various mutants might greatly simplify the methods of determinig whether diploid pollen grains occur, and their relative frequency in various parts of the plant. This assumption is not at all an improbable one when we recall the close association between the number of chromosomes and the size of the nucleus and the cell. I worked out these relationships in some detail in the case of *O. gigas* (Gates, 1909 a), and have since suggested (Gates, 1911a, p. 926) that the 4 lobes instead of 3 in the *gigas* pollen grain may result directly from the changed space-relationships which follow the doubling in the chromosome number. The 4-lobed grain undoubtedly contains more space, and therefore probably more cytoplasm, than 3-lobed grains, and it is reasonable to suppose that its nucleus is therefore larger, not only in *gigas* (as it undoubtedly is, though I have not made comparative measurements of the nuclei in mature pollen grains) but wherever 4-lobed grains occur. It is thus conceivable that the frequency of 4-lobed grains in such forms as *O. Lamarckiana* may be used as a measure of the frequency of diploid pollen grains. I should anticipate, however, that any diploid grains occurring in, e. g.,

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27) In a previous paper (Gates, 1911 a, p. 926) I gave Miss Lutz the credit for having first observed 4-lobed pollen grains, in *O. gigas*. But as a matter of fact I myself observed and figured such grains (1907) in my first paper on this subject, in a hybrid having twenty chromosomes.

Lamarckiana would be regularly quadrangular with 4 lobes, while triangular grains with an extra lobe would contain an intermediate number of chromosomes. In making calculations based upon the data given in my first paper on this phase of the subject (1909), I find this idea strongly borne out. The figures in the following Table are taken or deduced from the data given in that paper (pp. 531, 533), the nuclei being treated as spheres.

Table IV.

	Cytoplasmic volume	Nuclear volume	Ratio of increase of cytoplasm in *gigas*	Ratio of increase of nucleus in *gigas*
O. Lamarckiana . .	26995.39	1098.07	1.50 : 1	2.16 : 1
O gigas	39951.78	2373.52		

From this table it can easily be calculated that, if the cytoplasm in *gigas* pollen mother cells increased in the same ratio as the nucleus, it should measure in volume 49244.67. The deficiency in cytoplasm therefore amounts to about ·23, or nearly one-quarter of the amount of cytoplasm in the *gigas* pollen mother cell. While these figures are of course only approximately accurate, yet they make it at least reasonable to suppose that the fourth lobe in the *gigas* pollen grain serves to restore the normal ratio between nucleus and cytoplasm.

Another simple calculation from the data given in my previous paper (1909 a), shows that in *O. Lamarckiana* pollen mother cells in synapsis the ratio of cytoplasm to nucleus is about 24.58 : 1, while in *O. gigas* the same ratio is only 16.83 : 1.

With whatever degree of accuracy this relationship between extra lobes and increased chromosome number may be found to hold, it will be worth while keeping it in mind as a working hypothesis. Miss Lutz (1909, p. 266) makes the statement that in *O. lata* and *O. Lamarckiana* about one in a thousand grains have four or more lobes, "although as high as fifteen per cent. has been observed in normal, typical individuals". The latter statement requires verification. From the data presented in this paper, it is evident that plants having different types of pollen grains differ also in external characters and in chromosome number. It is highly probable that the plants of *Lamarckiana* referred to by Miss Lutz as having such a high percentage of quadrangular grains were in reality triploid mutants. She mentions the frequent occurrence of grains having 4 or more lobes in the triploid mutants described in her recent paper (1911, p. 389), where she found the 3-lobed

grains "much in excess", but has not determined the proportions of the various types. Stomps also mentions (1912 b, p. 533) that, in the triploid mutant he obtained in the F_2 from *O. biennis cruciata* $\times$ *biennis*, the pollen grains were frequently quadrangular. In the small-flowered plant of *gigas* Italy above, the pollen grains (see Table VI) were about 23 per cent. 3-lobed, 75 per cent. 4-lobed, and 2 per cent. with more than 4 lobes.

As a matter of fact there are different types of 4-lobed grains. The typical *gigas* pollen grain is quadrangular with a lobe at each corner of the square, while the typical *Lamarckiana* grain is triangular with a lobe at each corner of the triangle. But triangular lobes also occur having an extra lobe on one side of the triangle. It is not impossible that such grains will be found to have a chromosome-number between seven and fourteen, for they will contain less cytoplasm than a corresponding quadrangular grain. Even if the above-suggested relation between the shape of the pollen grain and the contained number of chromosomes should prove not to be a constant one, it might still be frequent enough to serve as a valuable indication of probable chromosome number and distribution during meiosis, as a preliminary to making crosses and a cytological study. That this is so will be evident from certain facts here presented, and in any case the association here suggested is at least true to the extent that 3-lobed grains seldom occur in normal *gigas* and 4-lobed grains seldom in normal *Lamarckiana*.

The data from my examination of pollen grains are presented in the following tables. They are incomplete, as I had only a limited time to devote to this work [28]), but a number of very interesting facts appear. In a more thorough study, the proportion of good to bad grains should be determined in all cases, and also the number of triangular and quadrangular among the bad grains, for it is not impossible that there may be selective elimination of one type as the pollen matures. It is to be hoped that other workers will include a statistical examination of the pollen grains in the description of the plants they study.

In the flowers of *gigas* Italy very little pollen was produced, and it was granular, rather than stringy as in other forms. The anthers also usually failed to dehisce properly, so that in making pollinations the pollen had usually to be dragged out with a pin. It should perhaps be added that these pollen examinations were all made within a few days of August 20th, in the midst of the flowering period.

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28) My only reason for publishing these incomplete data at this time is to enable other workers to combine this method of pollen study with the observation of other characters.

Table V.

Pollen grains of *O. gigas* and other races.

	Total number of grains examined	% of „good" grains	% of „bad" grains	„Good" grains			„Bad" grains		
				3-lobed	4-lobed	5- or more lobed	3-lobed	4-lobed	5- or more lobed
O. gigas Italy ($^{227}/_{12}$).									
a) No. I. 6 (normal plant), grains from one flower . .	1050	27.62	72.38	18	251	21	total = 760		
b) Another normal plant (pedigree number not recorded)	662	42.60	57.40	6	255	21	1	371	8
Totals	1712			24	506	42			
					572			1140	
c) No. I. 4 (small flowers) 1st flower	591	39.76	60.24	61	165	9	107	249	0
2nd „	1210	25.62	74.46	63	239	8	total = 900		
3nd „									
(Good grains only). Stamen (1)	—	—	—	50	85	3	—	—	—
„ (2)	—	—	—	15	28	0	—	—	—
„ (3)	—	—	—	24	108	4	—	—	—
„ (4)	—	—	—	23	143	0	—	—	—
Totals for 3nd flower	483	—	—	112	364	7	—	—	—
Totals for No. I. 4	2284	32.69	67.35	236	768	24		1256	
O gigas Sweden ($^{233}/_{12}$). II. 1 normal	948	36.81	63.19	33	306	10	total = 599		
O. Lamarckiana	910	57.6	42.4	910	0	0	—	—	—
O. semilata ($^{229}/_{12}$ I. 6)	1198	44.28	55.72	530	0	1	667	0	0

From the accompanying tables (V. and VI.) it will be seen that in typical *O. gigas* Italy a fluctuating amount of the pollen, probably in most cases much over fifty per cent. was non-functional. Of the grains having a normal appearance, from 2—6 per cent. were 3-lobed, 86 to 90 per cent. were 4-lobed, while about 7 per cent. had five or more lobes. In the individual (No. I. 4) having smaller flowers, leaves and stature, the amount of sterility is about the same, but the number of triangular grains was regularly about 25 per cent. in all the flowers examined (Table VI), grains having five or more lobes only numbering about 1.5—4 per cent. Thus in every case the ratio of 4-lobed to 3-lobed grains in this plant was found to be approximately 3 : 1. Whether this Mendelian ratio may have any significance as such is not at present clear. Its chief interest lies in the fact that the smaller size of the plant organs is accompanied by this peculiar behaviour of the pollen. A count of the chromo-

somes in this plant is being made from preparations made by Miss Nesta Thomas. I have thus far been unable to find more than 25 or 26 chromosomes, but we are continuing the work on this and other forms, of which a full account will be published later.

Table VI.

Percentages of pollen types among "good" and "bad" grains.

	Total grains examined	"Good" grains			"Bad" grains		
		3-lobed	4-lobed	5- or more lobed	3-lobed	4-lobed	5- or more lobed
O. gigas, Italy a) No. I. 6 . . .	1050	6.2 %	86.55 %	7.24 %	—	—	—
b) Another plant	662	2.13 „	90.43 „	7.45 „	0.26 %	97.63 %	2.11 %
c) No. I. 4 1st flower	—	25.96 „	70.21 „	3.83 „	—	—	—
2nd „	—	20.32 „	77.09 „	2.58 „	—	—	—
3nd „	—	23.19 „	75.36 „	1.45 „	—	—	—
All 3 flowers . . .	2284	22.96 „	74.71 „	2.33 „	—	—	—
O. gigas, Sweden No. II. 1	948	9.46 „	87.68 „	2.86 „	—	—	—
O. Lamarckiana .	—	100 „	—	—	—	—	—

In addition to the types of pollen grain already mentioned, I not infrequently found in *O. gigas* a more nearly globular type in which only two lobes were visible. No account was kept of these, as they varied much and were probably non-functional. The actual percentage of sterility is very difficult to determine, as shrivelled grains of all sizes occur, and I have long known from sections that many of the pollen mother cells also break down before or during the reduction divisions. Another peculiarity worthy of mention, is the way the various types of pollen grains appear to be grouped. Thus in the plant having about 25 per cent. of triangular grains, the latter were often found in groups on the slides, frequently several together, rather than equally distributed through the rest of the pollen. This would suggest that possibly their production was due to irregularities in the reduction divisions of certain mother cells, by which only the haploid number of chromosomes reached the daughter nuclei of the homotypic division. The same phenomenon was observed in normal *O. gigas* Italy (No. I. 6). In the examination of pollen from one anther 8 triangular grains were found together, and in another anther three triangular grains were observed quite close together on the slide.

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In *O. gigas* Sweden the amount of pollen produced is certainly much in excess of that in the Palermo race, though there is no evidence of a smaller percentage of bad grains in the meagre data of Table V. It appears also, from Table VI, that there are more 3-lobed grains and fewer having 4 lobes than in *gigas* Italy.

Turning now to the narrow-leaved *gigas* from the Amsterdam cultures ($^{180}/_{12}$), I found no good grains at all, though the anthers were fairly well filled with shrivelled grains, including several types I had not previously seen. The 4-lobed grains appeared most numerous, but their frequency, and that of the numerous 3-lobed grains, was not determined. In both these types the size of the lobes was large relative to that of the central part. In addition a rounded, nearly spherical grain with very small lobes was observed, but it appeared non-functional like the rest. The number of chromosomes in this narrow-leaved individual has not yet been determined, but I feel justified in· predicting that it will be less than 28. In fact, I believe the most probable hypothesis regarding the cause of the tremendous variation in leaf-width and other characters exhibited by *O. gigas* is to be found in variations in the chromosome-number of different individuals. This will be brought about by irregularities in meiosis, and the different types of individuals in the offspring of *O. gigas* will thus represent a partial return to the diploid number. It does not follow that all the size and shape relationships in these plants are to be explained in this manner.

In the flower of *O. Lamarckiana* examined (Table V) the number of good grains was found to be over fifty per cent., and the grains were all, both good and bad, 3-lobed without exception. I afterwards examined the pollen of several other flowers from different individuals, but without counting the grains, but I found not a single 4- or 5-lobed grain. I observed, however, that occasionally two grains lie over each other in such a way as to appear like one 4-lobed grain until carefully examined. Evidently 4-lobed grains must be very rare if they ever occur in my strain of *Lamarckiana*.

An individual of *semilata*, which is a well-defined mutant type, was examined. This plant (229. I. 6) occurred in a culture of *lata*-like plants from seeds kindly sent from Sweden by Nilsson ("Komb. 1"). One very large 5-lobed grain was revealed in the flower examined, but subsequent examination of another flower without counting failed to disclose another such grain, though a number of apparently 2-lobed grains were seen. A preliminary chromosome count of this individual, from preparations made by Miss N. Thomas, shows that the number is certainly as many as fifteen, and possibly sixteen. This form will also be reported upon more fully later, in connection with a paper on the various *lata* forms. I may say that another *semilata* plant from de Vries' seeds

originally, self-pollinated yielded this year only three individuals, of which two were typical *lata* and one *Lamarckiana*.

It is hoped that this method of pollen examination will be used in future by students of *Oenothera*, for it will evidently add much to our knowledge of the constitution of the plant.

Discussion.

In this section it is first necessary to devote considerable space to comments and criticisms of various statements made and conceptions held by other investigators.

To begin the discussion, I must first complete the paragraph from de Vries (1912, p. 34) from which the above sentence was quoted, which is as follows: „Zwar hat Gates eine abweichende Ansicht aufgestellt und behauptet, dass die Verdoppelung erst nach der Befruchtung geschehen sein sollte, und nicht einer Mutation (sic), sondern einem Zufall ('of the nature of an incident') zugeschrieben werden müsste. Dieses würde den Vorgang in die Gruppe der erworbenen Eigenschaften (sic) überführen. Und da man von diesen jetzt wohl allgemein annimmt, dass sie nicht erblich sind, genügt die Vorstellung von vornherein nicht, um die Entstehung meiner erblichen Rasse zu erklären. Ich erinnere hier an die doppelkernigen Zellen von *Spirogyra* in den Versuchen von Gerassimow, welche durch die vegetativen Teilungen hindurch ihre beiden Kerne beibehalten, diese bei der Befruchtung aber wieder verlieren. Übrigens wird die Ansicht von Gates durch die neueren Tatsachen völlig widerlegt."

This passage requires several comments. I have already pointed out that, so far from my view having been anomalous, it has been supported by nearly all the cytologists who have had occasion to deal with the subject. The second statement involves a misconception of my point of view. I have never suggested that the origin of *O. gigas* was not a mutation, but on the contrary, have held it to be such, since it results from an inherited germinal change. I have, however, held it to be phylogenetically "of the nature of an incident", and in this it probably agrees with many other mutations, though it is, of course, almost impossible to measure the phylogenetic value of any germinal change when it is viewed in such short perspective. The numerous cases of tetraploid species among plants, and less frequently among animals, show, however, that this condition is, as I have pointed out (1909a), of great evolutionary interest.

The next statement of de Vries, that the origin of *gigas* from a change occurring in the fertilized egg, or in the megaspore mother cell would place it in the category of "inheritance of acquired

characters", is obviously not in harmony with the usual biological conceptions. For that term in biological usage, means the occurrence of a somatic modification, and its subsequent reflexion back into the germ plasm. But a megaspore mother cell or a fertilized egg is germ plasm par excellence, and any chromosome-doubling occurring here is obviously a germinal change, and as such likely to be inherited. We may contrast with such a condition the one obtained experimentally by Němec (1910), in which, by treatment of root tips, a doubling in the number of chromosomes in various cells of the growing tip is obtained. There is obviously no possibility of such a change being passed on to the next generation, and as a matter of fact the tetraploid condition of these cells gradually disappears, although opinions differ as to how the return to the diploid number takes place. De Vries cites in comparison with *gigas* the binucleate cells of *Spirogyra* obtained by Gerassimow, which afterwards returned to a uninucleate condition. But the recent important work of the Marchals, already cited, in obtaining diploid moss gametophytes by wounding the sporophyte, is a closer parallel, and those experiments frequently gave rise to constant tetraploid races. The statement of de Vries, that my point of view is „völlig widerlegt" by the newer facts, is therefore scarcely in accord with the evidence.

De Vries (l. c., p. 35) calls the triploid forms half-*gigas* mutants and states that they agree in character with *O. gigas* × *Lamarckiana*, a result which would be expected. He finds that when *Lamarckiana* is crossed with pollen from *cruciata, muricata* or *Millersi* (nov. sp.), most of the seedlings produced are yellowish, the occasional deep green ones (15000 yellowish to 45 green), called Hero, having 21 chromosomes. This gives a mutation-coefficient of about 0.3 per cent., which is assumed with probability to represent the frequency of diploid eggs in *O. Lamarckiana*. This of course furnishes no evidence of diploid pollen grains. If such really occur in *Lamarckiana* and are functional, it would seem probable that their frequency might be determined by making the reciprocal crosses, with *Lamarckiana* as the pollen parent, but this does not seem to have been done. We must conclude, then, that the interesting evidence offered by de Vries shows only the frequency of diploid egg cells, the occurrence of which we already had some reason to believe in through the observation of Geerts, but offers no support whatever for the occurrence of functional diploid pollen grains.

In discussing the status of *O. gigas*, de Vries (l. c., p. 36) regards it, and I believe rightly, as „eine gute progressiv entstandene Art". He believes also that many of the differences from its parent, *O. Lamarckiana*, cannot be explained as a result of the original

chromosome-doubling and the various changes it entailed. This view may be correct but it has certainly not yet been proved, and I think it can be shown that most of the changes at least may have resulted from a single original change, namely the doubling in chromosome-number. De Vries cited the following characters as unexplainable on the basis merely of the increased size of the nuclei and cells: 1) The strong biennial habit. But this means merely a slower rate of development under given conditions, and Keeble's (1912) giant Primula also grew more slowly, a result which might be expected to follow directly from the larger size of the cells, and the slower rate of karyokinetic division. 2) The larger seeds. It is difficult to see why this was cited, for the ovule and hence the seed, is an organ which would obviously be larger if composed of larger cells, as the *gigas* ovule undoubtedly is. 3) The small (i. e., short) fruits. The explanation here is not so obvious, but the ovary at the time of fertilization is longer and stouter (see Table II, p. 119) than in *Lamarckiana*, again a direct result of the larger cells; and in *gigas* Sweden (see Table III, p. 126) the mature capsule is also longer than in *Lamarckiana*, while it is shorter in *gigas* Italy and in *lata*. After careful comparative study, the explanation of this is simple — the length depends upon the amount of sterility, or in other words upon the number of ovules which mature seeds. If anyone examines a nearly mature capsule of *gigas* or *lata* they will find a comparatively small number of seeds and a large number of undeveloped ovules. Part of these ovules doubtless fail to develope for lack of fertilization, but many of them (as I have learned from cytological studies of oogenesis in *lata*) fail to develope because the meiotic processes go awry. In *lata* the sterility from this cause appears to be even greater than in *gigas*. The short length of the mature capsule in both these forms depends upon the small number of seeds developed in them [29]), and is therefore easily explained without recourse to another mutational change. The greater sterility of ovules, both in *gigas* and *lata*, might be expected to follow from the meiotic difficulties introduced by a) the tetraploid, and b) an odd number of chromosomes. However, in

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29) There is a further interesting point, namely, that the seeds produced, particularly in *lata*, are scattered through the length of the capsule, with many undeveloped ovules between them. The latter do not produce seeds because they are incapable of being fertilized. On the other hand, when the fertilization of an ovary in *O. rubrinervis* and other forms is incomplete, through the failure of sufficient pollen to reach the stigma, one finds almost invariably that the l o w e r m o st ovules are the ones which are fertilized and develope seeds. Hence it appears that when all the ovules are capable of being fertilized, the first pollen tubes must grow to the b o t t o m of the ovary, the next to the ovules next above, and so on to the top. This behaviour cannot be explained by a summation of chemotactic influences from all the ovules, for in that case the middle ovules would be the first to be fertilized.

gigas Sweden the mature capsules are very long and contain many more seeds than in *gigas* Italy. But in this case the ovaries ready for fertilization are extremely long and probably contain more ovules, so that the percentage of sterility may be no less than in *gigas* Italy. De Vries cites (4) the outgrowth of the axillary buds on the stem to form branches, as another difference. But this is an extremely variable character both in *gigas, Lamarckiana* and the other forms, depending no doubt upon local environmental conditions at the time when these various buds reach a certain stage of development. It can therefore scarcely be considered a constant differentiating mark from *Lamarckiana.*

Another interesting physiological difference noted in the *gigas* races during the past season was their greater susceptibility to frost. On the morning (Oct. 5) after a rather heavy frost, the flowers and unopened buds of the *gigas* races were bitten and drooped over, while all the diploid races in the garden escaped. The only exception was the small-flowered plant of *gigas* Italy (227. I. 4), which was unaffected. This difference again is no doubt a result of the larger size of the cells in *gigas.*

Since it is possible to explain easily so many apparently diverse morphological and physiological characters in *gigas* as the result of a single initial change in nuclear structure and consequently in cell size, one must hesitate before affirming that any character of *gigas* is necessarily the result of another (additional) change. Our knowledge, or rather our ignorance, of morphogenesis is at least as profound as that of the physicist who cannot explain why a certain rate of ether vibration gives the sensation of red and another the sensation of blue; or as that of the chemist, who cannot correlate the properties, such as color and crystalline shape, of his compounds, with their chemical composition except in a very limited way. It is evident that many secondary changes in *O. gigas*, such as the larger seeds and shorter capsules; and physiological changes such as the stronger biennial habit and greater susceptibility to frost, follow as a result of an initial quantitative change in nuclear and cell structure. According to the logical "law of parsimony" one cannot introduce an additional cause to explain these changes unless they can not be explained without its help, but I have shown that the characters cited can be so explained. Regarding the few characters of *gigas*, such as leaf-shape, which remain to be explained, one can only say that in our present ignorance of morphogenesis — of the relation between organic cell structure and external form — we cannot really distinguish between quantitative and qualitative characters, except that we do know that in many cases specific differences which appear to be qualitative are found when analyzed to rest upon ultimate quantitative differences.

At any rate, the characters of *gigas* cited by de Vries as antagonistic to this view are seen to be readily explainable in harmony with it. Whether the leaf-shape requires the assumption of an additional correlated change, remains to be seen, but the case of *Phascum cuspidatum* shows that this is quite possible.

In *gigas* Sweden many of the characters are obviously different from those of *gigas* de Vries, but the parental race of *Lamarckiana* from which it arose was also different (see Nilsson, 1912), and until the chromosome numbers of both these races are known it is useless to discuss the exact status of this giant race, though many of its characters are obviously a result of the giantism of its cells.

The Chromosomes of *O. gigas* hybrids.

Without occupying too much space with unnecessary details, I may be permitted to refer to a few of the facts, chiefly cytological. Miss Lutz (1912) has devoted much of her recent paper to a discussion of the probable status of the triploid plants which formed the basis of my paper on chromosome reduction in *O. lata* × *gigas* (Gates, 1909 b). There must, unfortunately, remain some doubt as to the exact male parentage of these plants, but I think the facts on the whole certainly justify my treatment of them and I shall continue to refer to them as *O. lata* × *gigas*. By ingeniously bringing together quotations from several of my early cytological papers, regarding the foliage and bud characters of these plants, Miss Lutz has made statements appear contradictory which in reality are not so. In the early papers the descriptions were naturally less detailed than now when our knowledge concerning the various types is much more accurate. Furthermore, the papers were meant to be cytological rather than systematic. But anyone familiar with the characters of *Lamarckiana*, *lata* and *gigas* knows that the leaves, for example, of all three resemble each other in varying degrees, and it was obviously my purpose in the cytological papers mentioned, to refer merely to the general features of comparison. Since then, the necessity for very detailed studies of the external characters has become clear, and much of my time for several years has been devoted to the correlation of the cytological features with the external characters, only fragments of the results of which have yet been published.

I have also made the cross *lata* × *gigas* a number of times since 1907, but only once with success until this season. In 1909 I made the cross *Lamarckiana* × *gigas*, obtaining an F_1 of forty plants which were all identical with *Lamarckiana*. The pollen when examined contained only triangular grains. Two F_2 families were grown in 1911 and one F_3 in 1912, all giving typical *Lamarckiana*. This experiment is referred to in a paper now in press.

Certain plants of the F_1 were examined and found to have fourteen chromosomes. Of course there is the bare possibility that the wrong capsule was collected from the *Lamarckiana* plant in the original cross. But this seems improbable. Another explanation is that all the functional pollen grains of the *gigas* parent, owing to irregularities in meiosis, contained only seven chromosomes, or again as happens in certain Echinoderm hybrids, the extra chromosomes may have been extruded and lost from the nuclei in the early mitoses of the fertilized egg. Geerts (1911) found that in *Lamarckiana* $\times$ *gigas* in the F_2 the number of chromosomes returned to fourteen, but contends that the F_2 hybrids were still identical with those of the F_1, an observation which is open to grave doubt.

In my paper on meiosis in *O. lata* $\times$ *gigas* (Gates, 1909 b), I demonstrated clearly that in my material the twenty-one chromosomes on the heterotypic spindle regularly segregated into groups of ten and eleven chromosomes, with only occasional cases of a 9—12 distribution. Scores of nuclei were counted in interkinesis, and in every case the result was as above stated. No cases were observed of greater irregularity in the heterotypic distribution, and none were found in which chromosomes were left out in the cytoplasm during interkinesis. These results were established beyond the slightest doubt in my paper above mentioned, yet Geerts (1911) attempted to throw doubt upon them because, as he thought, his own results were incompatible with them. Another point, which was referred to by Strasburger (1910) and subsequently by Geerts (1911) was with regard to the possible paired arrangement of the twenty-one heterotypic chromosomes. Strasburger reproduced my figures 9 and 10 (plate XII) and 11 (plate XIII) as giving some evidence of such a paired arrangement, which they probably do. But I never found the pairing in the hybrid evident enough to be quite convinced of its significance, although I studied this point before my paper was published. As I first showed several years ago, the chromosomes are very loosely arranged on the heterotypic spindle, so that even in pure races of *Lamarckiana* forms and of *O. biennis*, the evidence of pairing at this stage is often very doubtful. Davis has since confirmed these results for (1910) *O. biennis* and (1911) *O. Lamarckiana*. Even assuming, as is not improbably the case (although I did not obtain thoroughly convincing evidence of it), that in the twenty-one-chromosome hybrid seven chromosome pairs are regularly formed, still the fact that the remaining seven chromosomes were almost invariably distributed in groups of 4 and 3, remains to be accounted for, as I pointed out (1909 b, p. 194). The fact remains that in my material the heterotypic mitosis was passed through with great regularity and uniformity. The homotypic mitosis also was completed with very few irregularities, and the

figures published proved this to be the case. The only irregularities discovered were 1) the passage of one chromosome to the wrong pole of the heterotypic spindle with about the same frequency as I had previously found this to occur in *O. rubrinervis* and other forms; and 2) the occasional omission of a chromosome from one of the daughter nuclei of the second division. My fig. 16 (plate XIII), which shows this, also shows that two of the nuclei contained ten chromosomes, while a third nucleus was cut. It is thus perfectly evident that my material showed remarkably few irregularities during reduction. Another interesting peculiarity, which Geerts (1911) first called attention to, but which is also evident from certain of my figures, is the failure of certain of the chromosomes to split during interkinesis. I had previously been inclined to interpret this as due to the wide variation which I had shown to exist in the time when the split of the homotypic chromosomes occurs. Geerts figures some of these unsplit chromosomes afterwards degenerating, but it is probable that some of them were in my material distributed to the homotypic daughter nuclei without dividing, for I rarely found chromosomes left in the cytoplasm, and never found any fragmenting, such as Geerts figures.

It is evident from such figures as Geerts (1911) publishes, that his results are in the main a confirmation of mine. The only differences are 1) perhaps a closer pairing of the homologous heterotypic chromosomes in the material studied by Geerts; 2) a tendency for the unpaired chromosomes to fragment or be left out of the daughter nuclei in the heterotypic telophase. Not a single case of this kind was to be found in my material. 3) Apparently greater irregularity, with fragmentation of chromosomes, in the homotypic mitosis. Thus it is obvious that the full number of functional chromosomes was retained throughout the reduction divisions in my material much more frequently than in that of Geerts. Whether this difference was due to the time of flowering, the particular weather conditions under which the meiotic processes were going on, or to some unknown difference in the hybrids, is not certain. But it seems probable that the more numerous irregularities in Geerts' material are to be attributed to the fact that his collections were made very late in the season, when the plants were nearly through blooming and the weather conditions must have been much less favourable. Miss Lutz (1912, footnote p. 405) states that his material was collected in September and October. My material was certainly collected much earlier in the summer, in the height of the flowering season.

Miss Lutz (1912) brings forward an imposing array of hypotheses to account for the various chromosome numbers now known in *Oenothera*, hypotheses based largely upon the observations of

myself, together with those of Geerts and Davis, on the pheno-
mena of meiosis in these forms. These interesting suggestions need
not concern us now, but they by no means exhaust the possibilities,
and at least one other cause of chromosome diminution is as likely
to occur as some that she mentions, namely, the loss of extra chro-
mosomes from the nuclei during the early divisions of the embryo.
I presented certain evidence for this earlier in the present paper.
The nuclear divisions of the male gametophyte are also a likely
place for loss of chromosomes to take place from an unbalanced
chromosome group. It is also conceivable that the two male nuclei
in a pollen tube might in this way come to have different chromo-
some numbers.

In connection with her discussion of the chromosome numbers
in Oenothera, Miss Lutz (p. 432) makes the extraordinary statement,
"So far as I have been able to discover, no mention has been
made of differences of chromosome number in mutants of *Oeno-
thera* previous to Gates' first paper. In this contribution he men-
tions no mutant with a chromosome number differing from that of
O. Lamarckiana." And in the following paragraph, "The first men-
tion of a mutant with a chromosome number differing from that
of *O. Lamarckiana* was published by the writer six months later".
From this the reader is left to infer that she (Miss Lutz) made
the first discovery of different chromosome numbers in *Oenothera*.
But it is probably well-known to every one, except perhaps Miss
Lutz, that the whole subject of chromosome numbers in *Oenothera*
was opened up by my paper (1907), in which it was clearly shown
that one plant had about fourteen chromosomes and another about
twenty. The first announcement of these results was made in my
paper read at the New York meeting of the American Association
for Advancement of Science, in December, 1906. And it is not
devoid of significance that Miss Lutz began her work with *Oeno-
thera* seedling root-tips in January, 1907 (as she herself admits,
1912, footnote, p. 389), i. e., within a few days of the original
announcement of my discovery.

Remarks on giantism in *Oenothera*.

In the previous sections of this paper I have dealt with various
gigas types now known in *Oenothera*, together with some of their
derivatives and hybrids. The variability of certain of these *gigas*
races has been described recently by Nilsson (1912), and by me
in a paper now in press. It is becoming obvious from facts regard-
ing the pollen grains, already mentioned in this paper, and also
from the chromosome numbers in the various *gigas* forms so far
as they have been determined, that the varying chromosome distribu-
tions in the giant forms are the real cause of many at least of

the extraordinary types which appear in their offspring. These chromosome numbers are being further investigated from material now in hand, and it seems evident that meiotic irregularities will supply the key to the cause of this extraordinary range of variation.

N. Heribert-Nilsson (1912), along with several Mendelian writers, adopts the fallacy that because a new type (mutation) varies in the later generations after its first appearance, therefore the original character-change was not single but compound in nature. This fallacy is particularly obvious in the case of *gigas*, where disregard of the established cytological facts leads Nilsson to a quite abortive attempt to explain the origin and later behaviour of the giant types. Thus he says (p. 178) „Einige soeben angeführte Tatsachen scheinen mir dafür zu sprechen, dass die Rieseneigenschaften, durch eine Kumulation von quantitativen Faktoren für Größe und Form, auf verschiedenen Individuen der Stammart verteilt, aufgebaut worden sind." By an hypothesis akin to Darwin's pangenesis he assumes that (p. 178) „innerhalb der verschiedenen Teile der Pflanzen Reihen von selbständig spaltenden quantitativen Einheiten vorliegen", and that these numerous independent factors all happen to meet together in a single pair of germ cells, to produce the giant type[30]). On this hypothesis, all the intermediate and aberrant types which appear in the *offspring* of *gigas* should appear rather in the same family with *gigas* itself, which is not the case. He does mention certain forms which he considers intermediate between *Lamarckiana* and *gigas*, namely his Komb. 8 (p. 129) which, judging from his description, is evidently a triploid mutant.

The application of his theory leads Nilsson into still further difficulties. Thus he says (p. 180) „Diese Tatsachen lassen sich aber durch die Annahme erklären, dass der *gigas*-Typus durch eine Plus-Addition von quantitativen und kumulativen Einheiten entstanden ist, welche erst in verschiedenen Organen *gigas*-Eigenschaften, aber nicht den *gigas*-Habitus aufbauen. Wenn dann diese Plus-Komplexe zufällig zusammentreffen, so entsteht die Habitusveränderung, die Mutante. Der *gigas*-Typus wäre also eine extreme und zusammengesetzte Plus-Kombination von Faktoren für Größe und Form zu betrachten[31])."

This assumption, which closely resembles de Vries' premutation hypothesis, therefore supposes that these numerous "factors" are at

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30) This hypothesis is put forward notwithstanding his admission (p. 219 and elsewhere) that in other cases one character-change can influence many organs, e. g. (p. 219), „die Eigenschaft der Rotnervigkeit. Diese beeinflusst nicht nur die Farbe der Blattnerven, sondern auch die Farbe, die Buckligkeit und die Größe der Blätter und die Länge der Früchte."

31) Italics his.

first cryptomeric[32]) but later, when they all meet together, suddenly make their presence felt. This hypothesis is too obviously contrary to fact to require comment.

If we regard the giant mutants as individuals in which the chief if not the only original change has been the sudden doubling in the chromosome number, we shall then be justified in looking upon such forms as progressive mutations. We should therefore expect them (as they do) to yield giant types parallel to the *Lamarckiana* mutants, such as *oblonga* and *scintillans*. The long range of narrow-leaved and aberrant forms which frequently appear in the progeny of *gigas* are, as I have already suggested, doubtless due to chromosomal aberrations and probably represent a gradual diminution towards the diploid number. The important point is that such forms occur only in the offspring of *gigas*, and not from other sources.

Nilsson's method of pure lines in his researches is greatly to be recommended and has led him to many valuable results. It is therefore all the more to be regretted that he has neglected the cytological facts in the interpretation of his results. What I wrote in 1907 (p. 108) is still true; "some process of differentiation (in *O. Lamarckiana*) the most probable seat of which is the germ plasm, has led to the production of distinct types of germ cells differing in chromosome morphology and in hereditary value." Nilsson, in discussing my view (p. 211) says, „Also ist Gates der Ansicht, dass Unregelmäßigkeiten in der Verteilung der Chromosomen bei der Bildung der Keimzelle die Ursachen der Entstehung von Varianten mit einer geringeren Anzahl von Eigenschaften als die der Stammart sind. Seine Auffassung unterscheidet sich also prinzipiell von der meinigen, nach welcher keine Unregelmäßigkeiten in der Bildung der Keimzellen stattfinden[33]), wenn die Verlustmutanten gebildet werden, sondern diese als Rezessivkombinationen durch eine Neukombination mendelnder Eigenschaften entstehen."

Since it is now well known that such meiotic irregularities as I described do occur, and are necessary to explain the origin and hereditary behaviour of such mutants as *lata* having 15 chromosomes (see Miss Lutz, 1912, and Gates, 1912) and any other mutants, such as certain *gigas*-forms, in which aberrant numbers of chromosomes occur, Nilsson's assumption must necessarily be discarded as contrary to fact. His attempt to show that (p. 213) „das ganze Mutationsphänomen dürfte unter einem gemeinsamen Gesichtspunkte: der Mendel'schen Neukombination, eingeordnet

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32) Nilsson himself uses this term (p. 160).
33) Italics mine.

werden können" has ended in failure, because it necessitates assumptions which the already known cytological facts disprove; though without a knowledge of these facts his hypothesis might have seemed plausible, at least in part.

N. Heribert-Nilsson (1912, p. 212) also attempts to explain the origin of *rubricalyx* from *rubrinerris* in my cultures through the accumulation in one individual, of several independent quantitative "factors" for pigmentation. He says, "*O. rubricaly.r* ging allerdings aus einem geselbsteten Individuum hervor, aber Gates erwähnt, dass er in seinen Kulturen mehrere *rubrinerris*-Linien gehabt hat, und Kreuzung zwischen ihnen kann ja in den vorigen Generationen stattgefunden haben".

Unfortunately for his theory, I can state the facts more definitely than they were given in my publication on the inheritance of pigmentation (1911 b). They are as follows: In 1906 I grew at Woods Hole, Mass., a culture of 45 plants from *rubrinerris* seeds of de Vries. These were all *rubrinerris* except two *Lamarckiana* and one *oblonga*. A number of the individuals were self-pollinated, and together with *rubrinerris* cultures from various other sources, making a total of over 1000 plants, were grown at the University of Chicago in 1907. The particular culture in which the *rubricalyx* individual appeared, contained 112 plants, all typical *rubrinerris* except the *rubricalyx* individual and one or two other rosettes which were somewhat aberrant and doubtful. This culture, which alone is concerned in the pedigree of *rubrinerris*, contained the offspring of four plants (Nos. 96, 98, 119 and 121) which had been selfed in the previous generation.

These four cultures should have been kept separate, but were thrown together because this made no difference in the experiments I then had in view. The *rubricalyx* mutant with its new dominant character therefore appeared as one of the 112 offspring of four purely self-pollinated *rubrinerris* individuals, which were sister plants from a culture of *O. rubrinerris* from seeds of de Vries. It therefore belonged to the second self-pollinated generation from de Vries' seeds, and any hypothetical crossing of pure lines must be relegated to de Vries' cultures. The presence of two *Lamarckianas* in the original culture may be attributed to mutation or to the entrance of foreign pollen, for I cannot be certain that the seeds is the original packet were guarded seeds.

Nilsson's hypothesis is impossible for several other reasons. 1) As I showed in the paper above-mentioned (Gates, 1911 b) from these very cultures, the range of pigmentation in *O. rubrinerris* buds was absolutely continuous, but there was a wide gap between the extreme plus variation in *rubrinerris* and the individual *rubricalyx* mutant (see plate 6 of that paper). 2) The behaviour

of the *rubricalyx* mutant showed that it was heterozygous, resulting from union between a mutated and a normal *rubrinervis* germ cell, because in later generations it has split into *rubricalyx* and *rubrinervis* in a 3 : 1 ratio. There were no intermediate types, and this fact together with the ratio are clear proofs of its origin as a monohybrid [34]).

Conclusions.

The purpose of the present paper is to consider giantism and tetraploidy in *Oenothera* from a comparative standpoint, in order to reach a better understanding of its nature and meaning in relation to evolution. Incidentally it has been necessary to refer to a number of other phenomena of mutation, and the main conclusions arrived at may be set forth as follows:

1. A survey of the species or races having tetraploid (4 X) or higher chromosome numbers, shows that stet. 30 such cases are now known in plants and 7 in animals, the about having been greatly increased in the last three years. On the other hand, not a single triploid wild species is known, although in some cases (e. g., *Drosera rotundifolia* $\times$ *D. longifolia* diploid and tetraploid species intercross, producing triploid hybrids. Triploid species can not be expected to occur in nature, since their chromosomes are not all paired and therefore (especially when the X number of chromosomes is odd) the meiotic processes will result in varying numbers of chromosomes in successive generations, leading to the sterility of many individuals, and finally to the gradual diminution of the chromosomes of surviving individuals to the diploid number.

2. The occurrence of triploid mutants in *Oenothera*, as shown by Stomps and Miss Lutz, is obviously due to the union of a diploid with a haploid germ cell. But this does not prove that the tetraploid mutant, *O. gigas*, originates from the union of two diploid germ cells of *O. Lamarckiana*. It is at least equally probable that *gigas* originates, at least in some cases, as I have suggested, from the apogamous development without fertilization, of an unreduced megaspore mother cell having (4 X) 28 chromosomes. This conclusion is based on a) the actual observation of such a megaspore mother cell in *O. Lamarckiana* by Geerts and the failure to observe the omission of reduction in the pollen mother cells, though thousands of times more of the latter than of the former have been studied. On the other hand, the occasional occurrence of 4-lobed pollen grains in *Lamarckiana*, points to the probability that diploid

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34) It is interesting to observe that Nilsson uses the occurrence of many types in the offspring of *gigas*, as an argument for its origin by the bringing together of many independent units, and yet applies the same reasoning to *rubricalyx* in whose offspring only two types appear in a simple monohybrid ratio.

pollen grains exist, though it does not prove that they are functional. b) An analysis of the facts in the bananas investigated by Tischler, which are sterile, seems to require that a single tetraploid cell developed a new individual and race by a mutation. And the Primulas, studied by Miss Digby, in which a single "pin" flower on one individual of the F_1 sterile hybrid between *P. floribunda* and *P. verticillata* gave rise in each of two independent crosses to a tetraploid race, makes it seem probable that the bud mutation which produced the "pin" flower was followed by normal fertilization and doubling of the chromosome number in the young embryo. In the case of the Mosses studied by the Marchals, it is obvious that tetraploidy arose through the aposporous development of a diploid gametophyte which afterwards produced diploid gametes which by fertilization gave rise to a tetraploid sporophyte; so that the essential change here was the production of a diploid gametophyte. This is emphasized by the fact that in one moss (*Phascum cuspidatum*) the apospory was accompanied by mutational changes in the new gametophyte.

3. The view which was held by myself, Strasburger, and others, that *O. gigas* and many other tetraploid species originated through a suspended mitosis just before or just after the formation of the egg, therefore remains to be disproved, and the facts seem to require this explanation at least in some cases. In any case, the evidence now at hand shows that in some plants the mutational changes are not confined to the meiotic divisions but occur also, 1) in the aposporous development of a gametophyte (the moss above-mentioned); 2) in bud mutations, such as the small-flowered and small-leaved branch of an individual of *O. gigas* Sweden described in this paper; 3) probably in an early division of the egg, in the cases of a periclinal and a sectorial chimera of *Oenothera* referred to in this paper.

4. In addition to the *gigas* of de Vries I have studied a race which originated independently several years ago at the Botanical Garden of Palermo, Italy, and whose characters are identical with those of the Amsterdam giant. A third giant race originated in the cultures of Nilsson at Lund, Sweden, from an independent Swedish race of *O. Lamarckiana* differing slightly from de Vries' type. This giant is, as Nilsson has shown, very distinct from the *gigas* of de Vries. A narrow-leaved descendant of the *gigas* of de Vries is also described in this paper.

5. A preliminary statistical study of the pollen grains in these giant races and in other *Oenothera* forms was made, and it was found that offspring of *gigas* which differed somewhat in their external characters differed still more strikingly in their pollen grains. Thus in normal *O. gigas* Italy there were about 28—43 per cent.

of "good" grains, and of these 6—2 per cent. were 3-lobed, 87—90 per cent. 4-lobed, while about 7 per cent. had 5 or more lobes. But one individual of this race was distinctly smaller in flowers, leaves, etc. possessed about 33 per cent. "good" grains, and of these 23 per cent. were 3-lobed, 75 per cent. 4-lobed, and about 2 per cent. 5- or more lobed. This individual probably contains less than 28 chromosomes. In the narrow-leaved *gigas* above mentioned, the pollen was all sterile, but 3-lobed and 4-lobed grains were both numerous.

In an individual of *gigas* Sweden examined, the amount of "good" pollen was found to be about 37 per cent., of which 9 per cent. were 3-lobed, about 88 per cent. 4-lobed, and about 3 per cent. with more than four lobes.

Since the triploid mutants also possess a certain (unknown) percentage of 4-lobed grains, it is obvious that there is a general parallel between the number of chromosomes and the percentage of quadrangular grains.

The percentage of quadrangular and triangular pollen grains is thus a very useful preliminary criterion to the approximate number of chromosomes possessed by a plant. It seems safe to conclude that all quadrangular grains contain more than 7 chromosomes, though it remains to be proven that they all possess the full 14. Probably triangular grains with an extra lobe possess an intermediate chromosome number.

It is shown by calculation from previous measurements, that in the pollen mother cells the ratio of increase of the cytoplasm in *gigas* as compared with *Lamarckiana* is only 1.5 : 1 while in the nuclei the ratio is 2.16 : 1. The deficiency of cytoplasm in the *gigas* pollen mother cells therefore amounts to about one-quarter and it is suggested that the extra lobe of the *gigas* pollen grain serves to restore the normal karyoplasmic ratio.

6. Several characters of *O. gigas* which were cited by de Vries as changes occurring independently of the chromosome-doubling, have been shown to be readily explainable as a direct result of the tetraploid condition with its larger cells and nuclei. These characters include (1) the strong biennial habit (2) the larger seeds (3) the short fruits (4) the greater susceptibility to frost. Whether any characters (such as leaf-shape) remain which can not be explained in this way, is a difficult question to decide in the present state of our knowledge of morphogenetic processes, but the effort should evidently be made, to explain the characters of giant types as far as possible on the basis of a single primary change, whether this be in chromosome number or merely in cell size.

7. A comparison of the observations of Geerts on meiosis in the pollen mother cells of *O. lata* × *gigas*, with my own, shows

that his material presented more irregularities in chromosome behaviour, which is probably due to his material having been collected very late in the season, while mine was collected in the height of flowering.

8. The various types of chromosome change in *Oenothera*, based on my own observations together with those of G e e r t s and D a v i s, are 1) in diploid races an irregularity in the distribution of the heterotypic chromosomes, resulting from the weak attraction between homologous chromosomes, 2) a similar irregularity (certainly much less frequent if it ever occurs) in the distribution of the halves of the homotypic chromosomes. The first of these is sufficient to account for the occurrence of 15-chromosome mutants, such as *lata*, or 16-chromosome forms. 3) The omission of chromosomes from interkinesis. 4) The union of a diploid egg with a haploid pollen grain. This is sufficient to account for the occurrence of all triploid mutants. 5) Possibly the latter may also arise by the union of a haploid egg with a diploid pollen grain, though there is at present no direct evidence for this. 6) Doubling of the chromosome number through a suspended mitosis in the megaspore mother cell, followed by apogamous development. This is sufficient to account for the origin of all tetraploid mutants, but they may also arise through 7) a suspended mitosis in the normally fertilized egg, or 8) the union of a diploid egg with a diploid pollen grain, though there are no direct observations in support of 7) or 8).

Chromosome numbers intermediate between the tetraploid, triploid and diploid may arise by crossing, and also 9) by irregularities in the heterotypic chromosome distribution in the tetraploid or triploid races, 10) by chromosomes being left behind in the heterotypic mitosis, 11) by chromosomes being omitted from the homotypic nuclei or fragmenting during meiosis. 12) By the failure of certain chromosomes to divide in the homotypic mitosis. To these we may add perhaps 13) the loss of chromosomes in the nuclear divisions of the male and female gametophytes, and 14) the loss of chromosomes during the divisions of the fertilized egg. All these processes except 2), 5), 7), 8), 13), and 14) are based on observations.

9. In the valuable paper of N i l s s o n he assumes that *O. gigas* has arisen through the accumulation of many independent quantitative factors for the size and form of various organs, an hypothesis which is inadmissible because it is contrary to the cytological facts as well as the facts regarding the sudden discontinous origin of the giant types and their s u b s e q u e n t wide variation. This variation is doubtless due in part to loss of chromosomes and in part to the fact that *gigas* contains the capacity of producing the same

mutant types as *Lamarckiana* itself. *O. gigas* is therefore, like
O. rubricalyx, a progressive mutant in the sense of d e V r i e s.

10. Hence although some of the characters in *Oenothera* are
Mendelian in their behaviour after they have arisen, yet Mendelian
combinations in the sense of N i l s s o n are wholly inadequate to
account for their first appearance.

11. I am, however, in agreement with N i l s s o n that many at
least of the mutant differences are due to changes which are fun-
damentally quantitative. Many of the size-differences in the races
with higher chromosome numbers are also probably due to differ-
ences in the number of chromosomes in their cells. It is the duty
of the observer to analyze these differences as far as possible in
terms of chromosome-number and cell-size. Evidently, any explana-
tion of the mutation phenomena in *Oenothera* which neglects the
cytological facts, is on very unsafe ground.

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On the Occurrence of Dextro-rotatory Albumins in Organic Nature.

By John Beard D. Sc., Edinburgh.

Twenty-four years ago the discovery of a transient nervous apparatus in the developmental histories of certain fishes directed my attention to the problems of alternation of generations in general, and of antithetic alternation in particular. Research in the intervening years was devoted mainly to the unravelling of the thread of animal development regarded as based in such an antithetic alternation of asexual and sexual generations. A review of the problems, which occupied the investigator during these years, and of their solutions, cannot be given here: the finds are recorded in the literature of science and most of the salient points may be found in detailed form in the memoirs, cited below[1]). These com-

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[1) Beard, J. The History of a Transient Nervous Apparatus in certain Ichthyopsida: An Account of the Development and Degeneration of Ganglion-Cells and Nerve-Fibres, Part I. *Raja batis*, in: Zool. Jahrbücher, Morph. Abteil., Vol. 8, with eight Plates, 1896.

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