

TWIN HYBRIDS OF *OENOTHERA HOOKERI* T. AND G.

HUGO DE VRIES

Lunteren, Holland

[Received December 24, 1917]

The researches of MORGAN on lethal factors, of BARTLETT on mass mutation, and of RENNER on empty seeds, have led me of late to the conception that lethal factors and hybrid mutants play a large part in the splitting phenomena which accompany the normal mutations of so many species of *Oenothera*. The question, which part of those phenomena may be explained by this view, has to be faced. The crosses of *Oenothera Hookeri* with the mutating species behave in so many respects differently from the remainder of the cases that they seem to afford suitable material for this study. *Oe. Hookeri* \times *Oe. biennis* produces a splitting in the second generation that does not follow the rules of MENDEL and *Oe. Hookeri* \times *Oe. Lamarckiana* produces twins of the type of *laeta* and *velutina*, but without their ordinary constancy, the *laeta* splitting in every generation into the same two types.

The discovery of mass mutation in *Oe. grandiflora* and of its relation to the production of twin hybrids by this species affords the clearest instance for this new conception and for this reason I shall describe in the first place the results of my crosses of this species with *Oe. Hookeri*.

CROSSES OF *OENOTHERA HOOKERI* WITH *OE. GRANDIFLORA*

My race of *Oe. grandiflora* was derived from a seed collected near Castleberry, Ala. It splits in every generation into two large groups. One is like the parent and repeats the splitting. The other is a weak form with pale broad leaves and a low stature. Most of its specimens die in early youth, some afterwards, but some reach the period of flowering and ripening their seeds. From these the pale race is reproduced pure. I consider this splitting, for reasons given elsewhere (DE VRIES 1917 b) as an instance of mass mutation and consequently call the aberrant type *Oe. grandiflora* mut. *ochracea*. Furthermore I assume this mutation to be the cause of the production of twins after crossing.

These appear in about equal numbers. The *laeta* come from the fertilization of the mutated gametes, but the non-mutated sexual cells produce the *velutina*. The proof for this view is given by corresponding crosses of mut. *ochracea*, since these do not produce a splitting but give a uniform progeny with the characters of *laeta*.

In *Oe. grandiflora* the male and the female gametes have the same hereditary qualities. This is shown by crosses with *Oe. mut. ochracea* and more directly by the fact that both reciprocal crosses with *Oe. Cockerelli* give the same twins. The same holds good for the crosses with *Oe. Hookeri*, as we shall presently see, and this is also an isogamic species.

I made both reciprocal crosses between my races of *Oe. grandiflora* and *Oe. Hookeri* in 1913 and sowed the seeds for a preliminary orientation in 1914 and for more detailed study in 1915. From these latter I derived the second generations in 1916. Moreover I repeated one of the crosses in 1915, in order to have a first generation for comparison with the second in 1916. These first generations consisted always only of two types, which could be sharply distinguished and counted at the time of flowering, in July and August. I found the following proportions:

TABLE I
First generation of *Oe. grandiflora* × *Oe. Hookeri*.

Cross	Year of cross	Number of specimens	Percent <i>laeta</i>	Percent <i>velutina</i>
<i>Oe. grandiflora</i> × <i>Hookeri</i>	1913	40	68	32
<i>Oe. grandiflora</i> × <i>Hookeri</i>	1915	69	40	60
<i>Oe. Hookeri</i> × <i>grandiflora</i>	1913	40	68	32
Mean			59	41

The figures come as near to equality as might be expected on the ground of the small size of the cultures. No other types appeared, and therefore one of them must be assumed to spring from the non-mutated gametes of *Oe. grandiflora* and the other from those which had mutated into *ochracea*.

The types were the same in the three cultures. The differences were small until the time of flowering. The *velutina* had long and narrow, more or less hairy leaves like *Oe. Hookeri* and its stems were dark brown. The *laeta* had pale brown stems with broader leaves with a wedge-shaped base and almost without hairs. They began to flower

about a week before the other twin. In August I measured some typical leaves, taking them at the same distance below the spike and found 2.5×14 cm for the *velutina*, but 3.5×15 cm for the *laeta*. Such a difference is very striking on the bed. The flowers were large and bright, being intermediate in all respects between those of the parents of the cross; they did not differ on the two twins. The dimorphism of the cultures could be seen clearly in the beginning of May, at the time of planting the seedlings in the garden, but at that period some individuals seemed still to be intermediate and to link the two groups together. During the growth of the stems, however, the differences became larger and more evident so as to make the limit between the two groups sharp and fully reliable. The cultures of 1916 have been compared with the offspring of the two types of hybrids of 1915 and wholly confirmed the estimation of the differential marks.

In 1915 I self-fertilized 8 specimens of *laeta* and 4 of *velutina*. The offspring of the latter were uniform in all characters with the exception of the size of the flowers. They embraced 70 individuals in each culture, one-half of which flowered in August. They all resembled their parents. No *laeta* and no aberrant forms were seen among them. Of the four parents two belonged to the combination *Oe. grandiflora* \times *Oe. Hookeri* and two to the reciprocal cross.

The offspring of the self-fertilized *laeta* offered a motley aspect. Various combinations of the characters occurred, but mostly only in a few individuals. Above these minor differences two types prevailed. One was that of the *velutina*, uniform among themselves and in all respects like the pure cultures just described. The other was evidently *laeta* and like the *laeta* of the first generation, but it was linked to the aberrant forms by so many transitions that it was often difficult to separate them. I had, however, no interest in studying these minor types, and so I relied only on the sharp limit between the *velutina* and the remainder. I counted them in the middle of August, when most of the specimens were in full flower or as near to this phase as was necessary to judge their spikes and flowerbuds. Some few plants had stayed in the condition of rosettes of radical leaves, but even here the differences were sharp enough for the counting.

I got the following results:

TABLE 2
Oe. grandiflora × *Oe. Hookeri*.
 Offspring of the *laeta* of the first generation.

Crosses of 1915		Total	<i>velutina</i>	Percent <i>velutina</i>	Mean
<i>Oe. grandiflora</i> × <i>Hookeri</i>	No. 1	66	33	50	39
	No. 2	65	18	28	
	No. 3	66	28	43	
	No. 4	65	22	34	
<i>Oe. Hookeri</i> × <i>grandiflora</i>	No. 1	57	15	26	42
	No. 2	66	35	53	
	No. 3	61	32	52	
	No. 4	66	23	35	

The seeds of these *laeta* do not contain any empty grains. I determined the number of germs in one hundred seeds for each of the hybrids of 1915 in the same lots of seeds from which the cultures of 1916 were derived and found for *Oe. (grandiflora* × *Hookeri) laeta* 94, 97, 98 and 100 percent of germs, and for *Oe. (Hookeri* × *grandiflora) laeta* 92, 93, 95 and 97 percent.

The total average of our table is 40 percent. This figure coincides almost exactly with that found for the first generation after the cross (41 percent, see above) and therefore would seem to point to the same cause. But into the cross between the two species *Oe. grandiflora* brings two kinds of gametes, whereas *Oe. Hookeri* has only one, and this condition was assumed to explain the equality of the two groups of hybrids, which the figures seem to indicate. The question arose, therefore, whether the *laeta* might have either in their pollen or in their egg cells only one kind of the hereditary qualities involved. This question may be answered by fertilizing the *laeta* with the *velutina*. This should then produce in one direction a splitting, but in the reciprocal cross a uniform progeny.

I made these crosses in 1915 and, in order to vary them as much as possible, took the twins from both the reciprocal crosses. I cultivated the offspring in 1916, counted them at the time of flowering and found the following constitution for the groups:

TABLE 3
Oe. grandiflora × *Oe. Hookeri*.
 Crosses of *laeta* with *velutina*.

Crosses	Total	<i>velutina</i>	Percent <i>velutina</i>	Mean
<i>Oe. (gr. × H.) laeta</i> × (<i>gr. × H.) velutina</i>	69	26	38	44
<i>Oe. (H. × gr.) laeta</i> × (<i>H. × gr.) velutina</i>	68	34	50	
<i>Oe. (gr. × H.) velutina</i> × (<i>gr. × H.) laeta</i>	65	31	48	48
<i>Oe. (H. × gr.) velutina</i> × (<i>H. × gr.) laeta</i>	70	34	48	

Total average 46 percent, or almost the same figure as in the two previous tables. Evidently there is no difference between the male and the female gametes of the *laeta*. And since both parents of the cross are themselves isogamic, there is no reason to expect a different behavior for their hybrids.

If our premises are right, we must get the same results when we replace the *velutina* in these crosses by the pure species. I made these combinations in the same year along with the others and counted the hybrids in the same manner. I got the following results:

TABLE 4
Oe. grandiflora × *Oe. Hookeri*.
Crosses of *laeta* with *Oe. Hookeri*.

Cross	Total	<i>velutina</i>	Percent <i>velutina</i>	Mean
<i>Oe. (gr. × H.) laeta</i> × <i>Hookeri</i>	69	46	67	70.5
<i>Oe. (H. × gr.) laeta</i> × <i>Hookeri</i>	71	53	74	
<i>Oe. Hookeri</i> × <i>Oe. (gr. × H.) laeta</i>	72	57	79	72.5
<i>Oe. Hookeri</i> × <i>Oe. (H. × gr.) laeta</i>	71	47	66	

This table confirms the results of the previous one, since the reciprocal crosses give about the same figures. The percentages for *velutina* are higher than in the crosses between *laeta* and *velutina*, but they seem to indicate the same process of splitting.

Our conclusion is, that the *laeta* hybrids of the reciprocal crosses of *Oe. grandiflora* and *Oe. Hookeri*, and all of them, split their male and female gametes into about equal groups of potential *laeta* and potential *velutina* and that the combination of these in fecundation produces about one-half of constant *velutina* and one-half of *laeta*, which repeat the splitting in the succeeding generations.

During the course of my investigations into the hybrids of *Oe. Lamarckiana* I have often observed that the size of the flowers is a character which is more or less independent of the other marks. So it is in this case also. In the first generation all the plants have large flowers, intermediate between those of the parents. In the second, however, much diversity is seen, on the beds of *laeta* as well as on those of *velutina*. Small-flowered specimens with flowers no larger than those of *Oe. biennis* appear and even some intermediate sizes may be found. For a closer study I chose the four groups of *velutina*, mentioned above, on account of their uniformity in all other marks, and counted their flowering specimens.

I found:

TABLE 5

<i>Velutina</i> from	Specimens with flowers		Total	Percent of small-flowered specimens
	large	small		
<i>Oe. grandiflora</i> × <i>Hookeri</i>	32	18	50	36
	29	12	41	29
<i>Oe. Hookeri</i> × <i>grandiflora</i>	30	4	34	12
	13	4	17	23
Totals	104	38	142	27

The average percentage of 27 coincides with the splitting of Mendelian monohybrids and indicates the small flowers as the recessive character. But none of the grandparents had small flowers. The most interesting point is, however, that the size of the flowers shows a splitting, whereas all the vegetative characters, which cause the *velutina* to resemble *Oe. Hookeri* so very much, are transmitted as a whole.

CROSSES OF *OE. GRANDIFLORA* WITH *OE. FRANCISCANA* BARTLETT

The high degree of affinity between *Oe. franciscana* (BARTLETT 1914) and *Oe. Hookeri* in external marks led me to the expectation that the behavior in crosses might also be the same. Both are Californian species with bright large flowers and long narrow leaves. I received artificially self-fertilized seeds through the kindness of Mr. BARTLETT, sowed them in 1915 and conducted my cultures so as to have annual plants. I fertilized a typical specimen of *Oe. franciscana* with the pollen of a plant of my race of *Oe. grandiflora* and cultivated in 1916 the first generation in 60 individuals, all of which flowered in July and August. They showed two types, analogous to the *laeta* and *velutina* of the previously described crosses. The foliage of the first was broad and pure green, that of the latter narrow and brown green. These differences slowly increased during the time of flowering, but remained small. There were 43 percent *laeta* and 57 percent *velutina*. The next year the second generation of the latter were uniform in respect to their typical marks, although there was some variation in minor characters, as e.g., in the size of the flowers, the rapid production of stems, and others. The offspring of the *laeta*, however, split into two main types. I counted among 60 flowering plants, 52 percent *laeta* and 48 percent *velutina*, both repeating the characters of the first generation. I have also made the reciprocal cross,

Oe. grandiflora × *Oe. franciscana*, but had only eleven plants of the first generation, which, however, showed exactly the same types as the first mentioned instance. There were 4 *laeta* and 7 *velutina*.

From these experiments we see that *Oe. franciscana* behaves in these crosses in the same manner as *Oe. Hookeri*.

CROSSES OF *OE. LAMARCKIANA* WITH *OE. HOOKERI*

Both reciprocal crosses between these two forms yield the twins *laeta* and *velutina*, but whereas these twins are constant in their progeny in other cases, the *laeta* from these crosses splits in its succeeding generations into *laeta*, which repeat the splitting, and *velutina* with a constant progeny (DE VRIES 1913, p. 131-132). Moreover, *Oe. Hookeri* as well as *Oe. Lamarckiana* are isogamic, carrying in their pollen and in their ovules the same hereditary qualities. The dimorphic groups do not contain their types in equal numbers, the average proportion being in the first as well as in the following generations, about 25 percent *laeta* and 75 percent *velutina* (idem, pp. 227-228 and 245-256). No constant *laeta* have been found. In consideration of some results with *Oe. mut. blanda*, which will be described elsewhere, I was especially interested in the question, whether the first generation would perhaps contain such, and would thus in reality embrace three types instead of two. Therefore I sowed in 1915 the seeds of a cross *Oe. Hookeri* × *Oe. Lamarckiana* made in 1909, got a culture of 81 specimens, among which 9 were *laeta*, retained only these and self-fertilized all of them. The next year (1916) I sowed the seeds, planted 60-70 specimens from each parent and counted their twins in June, when the plants were large rosettes of radical leaves showing their differences sharply. All of the nine groups were dimorphic with 5-11 *laeta* among a far larger number of *velutina*. The average percentage was 13. Some boxes were kept during the whole summer, in order to control the countings of June.

In the same lots of seeds I counted the germs in one hundred grains for each of the nine *laeta* of 1915. I found the following figures: 88, 90, 91, 91, 91, 92, 93, 94, 98, with a mean of 92 percent. From these numbers I conclude that these *laeta* do not contain hereditary empty seeds. The splitting is, therefore, to be considered as unilateral.

The phenomena afforded by these twins are exactly the same as in the case of *Oe. grandiflora* and therefore require the same explanation. The difference in the percentage figures does not seem to be essential, since in both cases the same figures were found for the first generation and for the offspring of the self-fertilized *laeta*. The figures for *Oe. grandiflora* indicate equality of the two groups, but in the case of *Oe. Lamarck-*

iana there is a loss on the side of the *laeta*. If we assume for *Oe. Lamarckiana* a mass mutation into *velutina*, analogous to that of *Oe. grandiflora*, but hidden by a linkage with the lethal factor which produces the empty seeds (DE VRIES 1918 b), exactly the same explanation of the twins holds good for both cases. In both, moreover, the *laeta* do not split after self-fertilization into three or more groups, according to the laws of MENDEL, but only into two. This fact, evidently, demands a special explanation.

All our suppositions are deduced from one point of view, a perfect analogy between *Oe. grandiflora* and *Oe. Lamarckiana* in the production of their twins, due to the same principal cause. *Oe. grandiflora* mut. *ochracea* and *Oe. Lamarckiana* mut. *velutina* do not differ from their parent species in one character only but in a whole set of such, one of which is the individual weakness in one case and the early death in the other. The absence of the parental type is common to both, but other properties differ. So, e.g., the leaves, which are broad in *ochracea* but narrow in *velutina*, smooth in the first but hairy in the second, and so on.

From this discussion it is easily seen that our hypothesis of a mass mutation and lethal factors is apt to simplify our conception of the twin hybrids and to bring them into causal relation with the phenomena of mutability in general.

For this reason it seems desirable to give here a review of the instances of constant and of splitting *laeta*. Constant *laeta* are produced by *Oe. Lamarckiana* and some of its derivatives (e.g., *Oe. brevistylis* and *Oe. nanella*) as well as by *Oe. grandiflora* after crosses with the female gametes of *Oe. biennis* and *Oe. syrticola*, with the male gametes of *Oe. biennis* Chicago, with both kinds of sexual cells of *Oe. Cockerelli*, and in some other instances. If we assume the gametes of *Oe. Lamarckiana* and of *Oe. grandiflora* to consist of two types, one of which produces the *laeta* hybrids and the other the *velutina*, we have to consider each of these combinations as a unisexual cross, the *laeta-velutina* character finding no antagonist in the other species. According to the conceptions, set forth in my book on the Mutation theory (German edition, Vol. II, p. 468) such unpaired characters produce constant hybrids, whereas Mendelian splittings require the assumption of an active and an inactive condition of the hereditary factors. This latter view has been changed, later on, by BATESON, into his hypothesis of presence and absence, which seemed a more empirical form of the same idea. But since MORGAN has shown that it is just as possible to determine the position of absent factors in the chromosomes of *Drosophila* as that of present units, it seems

to me that the question latency versus absence has been definitely decided in favor of the first alternative and that my hypothesis of the unisexual crosses is still unimpaired.

In the case of splitting *laeta* the sexual cells of the second parent must evidently contain a character antagonistic to that of *laeta* and this must be in the inactive or *velutina* condition. This is evident in the case of *Oe. blandina* (*Oe. Lamarckiana* mut. *velutina*) and must be assumed for the wild species *Oe. Hookeri*, in order to explain the complete analogy of the phenomena. The splitting *laeta* are, as a rule, provided with germs in almost all their seeds and this fact at once excludes the hypothesis of the influence of a lethal factor and of a third type of hybrids, hidden in empty seeds. It confirms the reality of a splitting into two types only.

I will now give a list of the observed cases, showing the percentage of germs in the seeds and of *velutina* among the offspring.

TABLE 6
Splitting of laeta into laeta and velutina.

Crosses	Percent of germs		Percent of <i>velutina</i>	
	in crossed seeds	in seeds of <i>laeta</i>	in crossed seeds	in seeds of <i>laeta</i>
<i>Oe. grandiflora</i> × <i>Hookeri</i>	85	97	46	39
<i>Oe. Hookeri</i> × <i>grandiflora</i>	94	94	32	42
<i>Oe. Lamarckiana</i> × <i>Hookeri</i>	95	92	89	65
<i>Oe. Hookeri</i> × <i>Lamarckiana</i>	96	85	78	69
<i>Oe. Lamarckiana</i> × mut. <i>velutina</i>	90	97	75	73
<i>Oe. mut. velutina</i> × <i>Lamarckiana</i>	95	95	52	71

The figures for the *velutina* of the first two crosses are deduced from the tables given above, those for the two following cases from my book on *Gruppenweise Artbildung* (1913, p. 132) and those for the last two from experiments described elsewhere (DE VRIES 1917 a). The percentages of germs in the crossed seeds have also already been published (DE VRIES 1916, pp. 251, 265, 270, and 1918 b). The *laeta letalis* are excluded from our table.

This table shows that the three cases run parallel, that the seeds do not contain empty grains due to the presence of a lethal factor, and that the *laeta* split off *velutina* in the same proportion as is observed in the first generation after the cross. In my book on *Gruppenweise Artbildung* I have deduced from the evidence given there that the *laeta-velutina*

qualities must be assumed to be present in the *Hookeri laeta* in a special condition (1913, p. 132), which, however, could not be that of a Mendelian hybrid, since constant *laeta* failed in my experiments. The same argument holds good for the *laeta* from *Oe. grandiflora* and from *Oe. mut. velutina*. The formula of MENDEL for monohybrids would require 25 percent *velutina* and if we would assume the presence of a lethal factor killing the germs in such a manner that their seed coats could not develop, it would lead us to expect 33 percent *velutina*. Our figures are, however, so much higher than these, that it is evident that even these assumptions could not explain them.

CROSSES OF *OE. LAMARCKIANA* WITH *OE. FRANCISCANA*

As already mentioned, this new species is so closely related to *Oe. Hookeri* that we may confidently expect analogous results from its different crosses. In order to control this deduction for this case I crossed some annual plants of my culture of 1915 with my races of *Oe. Lamarckiana* and *Oe. Lamarckiana mut. nanella*. The next year I cultivated sixty plants from each cross and almost all of them flowered in July-September. Already in April, when still in the boxes and before being transplanted into the garden, the young rosettes showed a difference. The larger part were almost like those of *Oe. franciscana*, but others were stouter and with broader leaves, resembling the *laeta* of the corresponding crosses of *Oe. Hookeri*. These differences increased gradually during the summer and were sharp and clear in August, without intermediates or dubious specimens. There were a large majority of *velutina*. I found:

TABLE 7
First generation.

Crosses	Percentage of	
	<i>laeta</i>	<i>velutina</i>
<i>Oe. Lamarckiana</i> × <i>Oe. franciscana</i>	24	75
<i>Oe. franciscana</i> × <i>Oe. Lamarckiana</i>	8	92
<i>Oe. franciscana</i> × <i>Oe. Lam. nanella</i>	5	95

In the first named culture a mutant occurred, which was an *Oe. oblonga* and became a large rosette, but did not develop a stem. Moreover in this culture about one-half of the specimens of *velutina* showed a pale yellowish tinge, which caused them to grow slowly and to produce only small, late flowering stems. In both the other cultures the *velutina* were dark green and as stout as the *laeta*. In this respect *Oe. franciscana* shows a complete parallelism with *Oe. Hookeri*, since the *velutina*, de-

rived from this species as a pollen parent, are also for a large part yellow and weak, whereas those from the reciprocal crosses are always dark green (DE VRIES 1913, p. 131 and p. 116, fig. 46).

Apart from this special feature the *velutina* of the three crosses exactly resembled one another, and so did the *laeta*. At the time of flowering they were one of the most beautiful groups of my gardens. The flowers of both twins are as large and as bright as those of *Oe. Lamarckiana*, the foliage of *laeta* is bright green, whereas that of *velutina* is brownish or almost red. The spikes and flower buds of the first are also green, but those of the latter are dark red. The differentiating marks were the same as in the corresponding hybrids of *Oe. Hookeri*. The leaves of the *laeta* were grass green and broad, those of the *velutina* dark green and narrow and more or less folded along their mid-vein. The spike of the first is loose, whereas on the latter the flowers and fruits are more densely crowded. The flower buds of the *laeta* are thin and have only some few red spots, but those of the *velutina* are thick and almost uniformly red. The pollen is pale yellow and spare in the first type, but in the second it is richly developed with long threads of viscin and almost white. The whole plants are glabrous in the first case, but hairy in that of *velutina*. From these and other marks we see that the similarity of these twins to those of other crosses of *Oe. Lamarckiana* is very striking.

I self-fertilized some specimens of each of the six types, sowed their seeds in 1917 and cultivated 58-60 specimens from each parent until the period of ripening their first seeds. I counted the parcels in August, when almost all the plants were in full flower, and got the following figures:

TABLE 8
Second generation of hybrids of Oe. franciscana.

Hybrids	Percentage of		
	<i>laeta</i>	<i>velutina</i>	Dwarfs
<i>Oe. (Lamarckiana</i> × <i>franciscana)</i> <i>laeta</i>	13	87	0
“ “ “ <i>velutina</i> ...	0	100	0
<i>Oe. (franciscana</i> × <i>Lamarckiana)</i> <i>laeta</i>	27	73	0
“ “ “ <i>velutina</i> ...	0	100	0
<i>Oe. (franciscana</i> × <i>Lam. mut. nanella)</i> <i>laeta</i> .	21	79	0
“ “ “ <i>velutina</i>	0	90	10

The dwarfs of the last cross bore all the characters of the *velutina*, apart from their nature. There were two mutants; viz., one *lata* in the second, and one with linear leaves in the third instance. We see from

this table, that the offspring of the *velutina* is uniform, but that that of the *laeta* splits into the same two types as were observed in the first generation and in almost the same numerical proportions. The dwarfs, which were absent in the first generation, reappeared in the second generation from the third cross, but only in that of the *velutina* and not among the seedlings of the *laeta*. In all these respects the crosses with *Oe. franciscana* exactly duplicate those with *Oe. Hookeri* and thereby give a confirmation of their results.

CROSSES OF *OE. HOOKERI* WITH *OE. BIENNIS*

The fecundation of *Oe. biennis* L. with the pollen of *Oe. Hookeri* gives uniform hybrids, which are constant in their progeny (DE VRIES 1913, p. 70). The reciprocal cross, however, is one of the few cases among the *Oenotheras*, in which the first generation is uniform, but the second is dimorphous (*idem*, p. 140). The first generation has the typical form of *Oe. biennis* but with the red color of *Oe. Hookeri* and was called therefore *rubiennis*. It splits into the same type and another which resembles *Oe. Hookeri* almost wholly. The *rubiennis* repeats the splitting in the succeeding generations, but the progeny of the *Hookeri*-like hybrids remains uniform.

No specimens of *rubiennis* with a constant progeny have been found. This absence distinguished the pedigree thoroughly from the scheme for a Mendelian monohybrid combination and the difference is enhanced by the fact that the splitting does not affect a single unit, but almost the whole group of the differentiating characters of the two species. The high interest attached to this problem has induced me to control the absence of constant hybrids of the type of *rubiennis* once more. With this view I have sown in 1915 some pure seeds collected in 1909, partly on biennial plants of the first generation, mentioned in my book for 1908, and partly on some annual specimens of the second generation. Every plant was fecundated purely by myself with its own pollen and their seeds were sown in pans and transplanted into boxes in 1916. Every box contained 60 seedlings. In April the difference between the *rubiennis* and the *Hookeri*-like was evident but some doubtful specimens remained. There was a large majority of the narrow-leaved form and some few specimens of *rubiennis*. These, however, were seen in every single box, and therefore among the progeny of each of the parents of 1915. No uniform set was found. I cultivated the boxes until the middle of June, when the rosettes became very stout with about 15-20 leaves; the full-grown leaves reached 15 cm. Those of the *Hookeri*-like

were narrow with pointed tips. All in all I had self-fertilized 22 plants, 11 of the second and 11 of the first generation. The percentages varied between 2 and 12 and were on the average 6 for the first and 4 for the second group. These are rather very low figures, even if they are compared with those of previous years (10-22 percent, see DE VRIES 1913, p. 103) but the summer of 1915 had been in many respects unfavorable for my cultures. The main point, however, was not weakened by this circumstance, since the question to be answered was, whether all specimens of *rubiennis* would produce *Hookeri*-like hybrids among their progeny, or whether there would be some without this splitting. The latter was evidently not the case.

We thus find the conclusion confirmed that in this case the splitting of the hybrids is unilateral. The explanation given in my book is thereby strengthened. It reposed on the heterogamy of *Oe. biennis*. The hereditary qualities of the male gametes of this species, or at least the majority of them, cannot be transmitted to the female sexual organs, neither in self-fertilization nor in crosses. Thus the hybrid *Oe. Hookeri* \times *Oe. biennis* carries in its egg cells only the characteristics of the pistil parent, but in its pollen those of both parents. If we assume that the latter are separated in synapsis into two groups and equally distributed over the pollen grains, we have one-half of the grains with the qualities of *Oe. Hookeri* and one-half with those of *Oe. biennis*. The first must evidently produce, in fecundation, plants of the *Hookeri*-like type, the second renew the cross and give hybrids with the qualities of *rubiennis*. It is easily seen, that the same deduction holds good for the succeeding generations. Control experiments have justified this conception. The question, however, why the two types never arise in equal numbers, but always with a large majority on the side of the *Hookeri*-like, remains still to be answered. But analogous deviations are often seen after crosses between species and mutants of the *Oenotheras*.

As usual, some mutants, especially of the type of *nanella*, were found in these cultures.

The conception of the heterogamy of *Oe. biennis*, from which our explanation started, may be controlled by experiments, in which the pure species is replaced by its hybrid with another heterogamic form. As such I have chosen *Oe. syrticola* Bartlett (*Oe. muricata*) and fertilized the reciprocal hybrids, *Oe. biennis* \times *Oe. syrticola* and *Oe. syrticola* \times *Oe. biennis*, by the pollen of *Oe. Hookeri*. If the hereditary characters of the male gametes cannot be transmitted to the female sexual cells, it is evident that the hybrids must behave, in these cases,

exactly like the pure species, which served as their female parent. In other words the characters which they inherited from their male parent, must disappear in the new cross. Of course, heterogamy is hardly ever absolute and does not necessarily embrace all the visible marks, and our argumentation applies only to the really heterogamous characters as such.

I made the crosses in 1913, using the hybrid races of the heterogamous species mentioned above, which have been described in my book on *Gruppenweise Artbildung* (p. 39-51). In 1914 I had from each of the two crosses a culture of 60 plants, among which 15 were allowed to flower and ripen their fruits. They reached a height of about two meters and I compared them, during their whole lifetime, with the hybrids between the pure species and *Oe. Hookeri*.

The results were as follows:

TABLE 9

Crosses	Type of hybrids
<i>Oe. (syrticola × biennis) × Hookeri</i>	<i>Oe. biennis × Hookeri</i>
<i>Oe. (biennis × syrticola) × Hookeri</i>	<i>Oe. syrticola × Hookeri.</i>

Each of the two groups was uniform, and exactly like the corresponding binary hybrid. It was evident that the characters of the central parent of the formula, as I called it, were as fully eliminated as is the case in the double reciprocal crosses between these two heterogamous species.

Of course, it is not necessary to use *Oe. Hookeri* as a male parent for these experiments. The pollen of other species may be chosen as well. Therefore I made some further crosses in 1913, using the same hybrid races, and cultivated next year 60 plants from each cross, in the same way as for the crosses with *Oe. Hookeri*. Here also the hybrids could be compared with the binary ones and the result was a complete likeness. Moreover, the cultures were uniform, as was to be expected. I got the following results:

TABLE 10

Crosses	Type of hybrids
<i>Oe. (biennis × syrticola) × Oe. biennis Chicago</i>	<i>Oe. biennis × Oe. biennis Chicago</i>
“ “ × <i>Oe. Cockerelli</i>	<i>Oe. biennis × Oe. Cockerelli</i>
<i>Oe. (syrticola × biennis) × Oe. biennis Chicago</i>	<i>Oe. syrticola × Oe. biennis Chicago</i>
“ “ × <i>Oe. Cockerelli</i>	<i>Oe. syrticola × Oe. Cockerelli</i>

These experiments confirm the results of those with *Oe. Hookeri*.

Excepting the hypothesis of merogony of R. GOLDSCHMIDT (1912), which has been refuted by RENNER (1913), no explanation of the heterogamous condition of *Oe. biennis*, *Oe. syrticola* and some other species has been proposed as yet. If it were allowed to apply our conception of the secondary mutability of hybrid mutants in this case, we might assume the initial mutations, by which these species arose, to have embraced changes which differentiated the sets of paternal and maternal characters. If then a hybrid mutant were produced, it would make two kinds of sexual cells in every generation. It would then be necessary to assume further that the gametes of one set were eliminated in the production of the pollen, and those of the other set in that of the egg cells. This would give constant heterogamous species just as our experiments show them to be constituted. Lethal factors would then have to be made responsible for these eliminations, but they would have to do their work before, and not, as in other cases, after fecundation. But it must be left to further researches to give an answer to these problems.

CROSSES OF *OE. FRANCISCANA* WITH *OE. BIENNIS*

Even as in the case of the crosses of *Oe. franciscana* with *Oe. Lamarckiana* the strong affinity of the former species with *Oe. Hookeri* must lead us to expect a similar behavior in its crosses with *Oe. biennis*. These have been studied already by DAVIS (1916, p. 217), who found a high degree of variability among their offspring and described a number of types. Among them a large majority were "franciscana-like," whereas another type was *biennis*-like. My cultures confirmed this result and showed that these two types are the main ones and that the numerous other combinations are only the effect of minor marks and can, with a few exceptions, be considered as belonging either to the "franciscana-like" or to the "biennis-like" group. Moreover this latter group duplicated the characters of the hybrids of the first generation.

The original cross for my cultures was made in 1915 and the first generation consisted in 1916 of 60 plants, all of which showed the same type. Forty-eight were biennials and did not make a stem, but the remaining 12 flowered abundantly during August. They were all like *biennis* in almost all their characters, but the leaves were narrower, their color paler, the spikes less dense, the tubes of the calyx longer and the flower buds thinner. At the time of flowering they resembled the hybrids of *Oe. Lamarckiana* × *Oe. biennis* more than the pure *Oe. biennis*. In all these points the influence of the pistil parent was evident and the

type could be described as intermediate between the two parents with a strong predominance of the pollen parent. The mid-veins of the leaves were reddish as in *Oe. biennis*, and on this ground we may designate the type by the same name as in the case of *Oe. Hookeri*, and call it, for convenience, *rubiennis*.

I self-fertilized four specimens, cultivated their offspring in 120 specimens for each of them and counted these in July, at the beginning of the flowering period, when the differences between the two main types were sharp and clear. About one-half of the plants had made stems but the others were still in the condition of rosettes. In both groups, however, the sorting out of the types left almost no dubious specimens. The majority of the plants were *franciscana*-like, as described by DAVIS, whereas the remainder resembled the first generation. I found:

TABLE II
Second generation of *Oe. franciscana* × *Oe. biennis*.

Parent	<i>rubiennis</i>		<i>franciscana</i> -like		Total	Percentage of	
	annual	biennial	annual	biennial		<i>rubiennis</i>	<i>franciscana</i>
No. 1	56	31	3	19	189	20	80
No. 2	49	32	7	20	108	25	75
No. 3	76	18	4	14	112	16	84
No. 4	40	53	4	14	111	16	84
Mean						19	81

The beds were inspected also during August and September, when in full flower and with ripening fruits, but the result was the same.

As was to be expected from the behavior of the crosses of *Oe. Hookeri*, the reciprocal cross did not give this splitting in the second generation.

If we compare these figures with those given in my book on *Gruppenweise Artbildung* (p. 103) for the second generation of the hybrids of *Oe. Hookeri*, we find an almost exact parallelism, since the figures for *rubiennis* were in that case 15, 20 and 22 percent.

CROSSES OF *OE. LAMARCKIANA* MUT. *VELUTINA* WITH *OE. BIENNIS*

From the considerations, given above, it may be deduced that *Oe. Lamarckiana* mut. *velutina* (syn.: *Oe. blandina*) must behave in an analogous manner in its crosses with the pollen of *Oe. biennis*. Therefore I examined this case, but only to such an extent as seemed necessary to verify the conclusions. I made the cross in 1915, sowed the seeds in 1916 and had a uniform lot of hybrids, which bore the type of *Oe.*

Lamarckiana × *biennis* (DE VRIES 1917 c). Among these I self-fertilized two specimens and found among their offspring, 24 and 31 percent *rubiennis* and 76 and 69 percent *blandina*-like hybrids. The former exactly resembled the first hybrid generation, the latter duplicated the pistil parent of the original cross.

Summarizing our results, we have for the crosses with the pollen of *Oe. biennis* the following mean constitution of the second generation.

TABLE 12
Hybrids of Oe. biennis in the second generation.

Cross	Percentage of	
	<i>rubiennis</i>	Type like pistillate grandparent
<i>Oe. Hookeri</i> × <i>Oe. biennis</i>	19	81
<i>Oe. franciscana</i> × <i>Oe. biennis</i>	19	81
<i>Oe. Lam. mut. velutina</i> × <i>Oe. biennis</i>	27	73

As in so many analogous cases the figures for the *velutina*-like offspring are higher than 50 percent. This shows that the splitting does not go into equal groups.

CROSSES OF *OE. HOOKERI* WITH *OE. SUAVEOLENS*

Crossed with different species, *Oe. suaveolens* Desf. gives, as a rule, uniform and constant hybrids. So, e.g., *Oe. biennis* × *Oe. suaveolens* and the reciprocal combination, *Oe. syrticola* × *Oe. suaveolens*, and *Oe. biennis* Chicago × *Oe. suaveolens* (DE VRIES 1918 a). Moreover the hybrids of *Oe. Lamarckiana* × *Oe. suaveolens* are uniform, whereas the reciprocal cross gives the usual twins. In all these respects *Oe. suaveolens* is analogous to *Oe. biennis* and the question arose whether it would also be so in its behavior towards *Oe. Hookeri*. I studied both the reciprocal combinations but cultivated for *Oe. Hookeri* × *Oe. suaveolens* only one generation, which I found strictly uniform. I made this cross twice, in 1913 and 1915, and had in 1914 a progeny of 90 and in 1916 one of 80 individuals. All of the latter and 25 of the former have flowered. They were in many respects like *Oe. Hookeri*, with reddish, narrow, hairy leaves of an intermediate shape and with the large bright flowers of this species, but still more narrowly resembled the hybrids of the reciprocal cross.

The results of this reciprocal cross, *Oe. suaveolens* × *Oe. Hookeri*, are complicated by the high degree of mutability of the first-named species, as compared with that of *Oe. biennis*. Otherwise they have

been exactly the same as those described above for *Oe. Hookeri* \times *Oe. biennis*. Therefore I shall use here the same names of *rubiennis* and *Hookeri*-like for the hybrids. The first generation was uniformly *rubiennis* but in the second and third generation the *Hookeri*-like were split off in proportions, which indicate a splitting into nearly equal parts. The figures were 53 percent in the second and 31 percent in the third, the latter figure being an average of the offspring of eight individuals of the second generation, all of which showed the splitting.

As already said the purity of this pedigree was diminished by the appearance of deviating types, which were evidently due to the mutability of *Oe. suaveolens*. In the first generation two narrow-leaved specimens appeared, and in the second and third, plants were seen with the pale broad leaves of the mutant *lutescens* (DE VRIES 1918 a), but evidently hybrids between this form and *Oe. Hookeri*. The progeny of both these types of mutants was uniform, as far as investigated. Some other deviations were observed, but not closely studied.

Oenothera suaveolens is heterogamous in many respects, and so the same explanation, as given above, may be applied to this case.

I will now describe the details of my experiments.

I made the cross in 1913 between two annual individuals of my races, and had in 1914 a first generation of 60 specimens, all of which grew to a height of 1 meter. Most of them flowered in August. They were a uniform lot with the exception of two narrow-leaved plants. They were evidently intermediate between the parent and very similar to the hybrids of the reciprocal cross. They combined the broader leaves of *Oe. suaveolens* with part of the reddish tinge of *Oe. Hookeri*. The two narrow-leaved specimens resembled *Oe. Hookeri* in this character and in the general color, but had broader and shorter flower buds, hairy leaves and weak stems with some few branches. Their leaves had only half the breadth of those of the typical plants, with the same length. They were probably due to the fecundation of egg cells of *Oe. suaveolens*, which had mutated into *fastigiata* or *jaculatrix* (DE VRIES 1918) or some other narrow-leaved mutant.

I cultivated a second and third generation of this narrow-leaved form and found them uniform in all respects, save the flowers. In 1915 twenty specimens flowered, among which 10 had the large flowers, common to both parents but the 10 others had very small petals, of 1.5 cm in length. This size is about the same as for *Oe. biennis*. I fertilized three large-flowered specimens, and found their progeny uniform in 1916 with 55, 66 and 42 flowering plants. I also fecundated one small-

flowered individual but the progeny of this one split in 1916 into 43 small- and 16 large-flowered plants. This points to a Mendelian monohybrid proportion with the small size as a dominant character. I might adduce that among the *Hookeri*-like hybrids of the splitting branch of the pedigree I fecundated in 1915 a large-flowered and a small-flowered specimen and found both constant in their progeny (36 and 53 plants in 1916). If it is allowed to combine all these data we have a complete set of the three types required by the Mendelian formula, viz., constant large and constant small flowers and splitting small-flowered plants, repeating their mark in about three-fourths of their progeny. But I have not further pursued this theme, my aim being only to prove that the size of the flowers varies independently of the other characters, which are always distributed by groups. The same dimorphy in the flowers was seen in 1916 on all the beds from seeds of the typical hybrids, but here the large flowers prevailed.

In compliance with the terminology used above, I shall here call the typical hybrids of the first generation *rubiennis*. After self-fertilization they split into *rubiennis* and *Hookeri*-like hybrids. My culture embraced 60 plants; they showed the dimorphy in the beginning of July, and developed their differences during the flowering period. The *rubiennis* repeated the marks of the previous year, but the *Hookeri*-like strongly resembled the two narrow-leaved plants, described above. There were 53 percent *Hookeri*-like. Among the others I found three plants, which unfolded the characters described elsewhere for the mut. *lutescens* (DE VRIES 1916 a, p. 7) and a slender specimen with smaller leaves. After self-fertilization each of them yielded a uniform progeny, which embraced 60, 57 and 39 plants for the three *lutescens* and 39 for the deviating type. This latter was a strikingly different combination of characters, having low, slender and richly branched stems with smooth small leaves, slender spikes and narrow flower buds of a pure green, large petals (3 cm) and the stigmata high above the anthers. The leaves reached in every direction about half the size of those of the *rubiennis*, the stems about half their height. There was almost no individual fluctuation in this group.

In 1915 I had self-fertilized 8 specimens of *rubiennis*. Their progeny embraced about 45-65 plants for each of them and consisted in almost every case of three types, viz., *rubiennis*, *Hookeri*-like and *lutescens*.

I found in August 1916 the following figures:

TABLE 13

<i>rubiennis</i> parent plant	Total	<i>rubiennis</i>	<i>Hookeri</i> - like	<i>lutescens</i>	Percentage of <i>Hookeri</i> - like
No. 1	56	31	20	5	36
2	60	40	17	3	28
3	61	25	29	7	48
4	64	27	11	26	18
5	47	23	20	4	43
6	46	26	20	0	43
7	50	41	5	4	10
8	56	28	13	15	23

This gives an average percentage figure for the *Hookeri*-like of 31 and for *lutescens* of 8. Besides these types there were found in No. 7 eleven plants of a new type (not counted in our table). This was a pure *aurea*, with broad golden leaves sharply contrasted with the remainder, even with the pale *lutescens*. They were transplanted in June to another bed, where eight of them flowered. The golden color diminished here, however, and the leaves became more markedly green, insuring the nourishment of fruits and seeds. But the type remained an obviously new one. Its appearance in about 18 percent of the whole culture evidently points to a Mendelian recessive and to an origin from a mutated sexual cell producing in the second generation a half mutant, analogous to those of *Oe. gigas nanella*.

If we combine all these data into a pedigree, we get the following summary:

TABLE 14
Pedigree of *Oe. suaveolens* × *Oe. Hookeri*.
Cross of 1913.

Cross 1913	1st generation 1914	2nd generation 1915	3rd generation 1916
<i>Oe. suaveolens</i> × <i>Oe. Hookeri</i>	{ 2 narrow-leaved.	uniform	uniform
		{ 3 <i>lutescens</i>	uniform
		{ 1 small-leaved.....	uniform
		{ 53 percent <i>Hookeri</i> -like	uniform
	{ <i>rubiennis</i>	{ <i>rubiennis</i>	{ 11 <i>aurea</i>
			{ 8 percent <i>lutescens</i>
			{ 31 percent <i>Hookeri</i> -like
			{ <i>rubiennis</i>

Leaving the narrow-leaved, small-leaved, *aurea* and *lutescens* out of consideration, as probably due to mutations, this pedigree coincides with

those given in my book (1913) for the three splitting types of hybrids of *Oe. Hookeri* \times *biennis*, *Oe. biennis* Chicago \times *Hookeri* and *Oe. cruciata* (*atrovirens*) \times *Hookeri*.

For the two latter it was assumed that the pollen lacks the heterogamous characters of the pistil parent and carries mainly those of *Oe. Hookeri*. If it is allowed to apply this conception to our cross, it must be possible to replace the pollen of the *rubiennis* in fecundation by the pollen of pure *Oe. Hookeri* or of the *Hookeri*-like hybrids of our pedigree without changing the result. Therefore I made these crosses in 1915. In both cases the progeny was the same as that of self-fertilized *rubiennis*, with the exception that deviating (mutated) forms failed and that the percentage for the *Hookeri*-like was rather high. It was 73 percent among 70 offspring of *Oe. rubiennis* \times (*suaveolens* \times *Hookeri*) *Hookeri*-like and 60 percent among a progeny of 68 individuals from the cross *Oe. (suaveolens* \times *Hookeri)* *rubiennis* \times *Oe. Hookeri* T. and G. These results justify the assumption made concerning the pollen of the *rubiennis* hybrids.

Many special features of these experiments require a further investigation, but the main result, viz., the splitting in succeeding generations, seems above all doubts. In combination with my previous researches it indicated a latent quality of *Oe. Hookeri*, different from allied species, which must be made responsible for the special splittings observed after so many crosses of this species.

In 1917 I have continued the culture of the new mutant *aurea*, in order to see whether this character was constant. I found it to be so. I sowed the seeds of three self-fertilized specimens of 1916; they yielded about 600 seedling plants, all of which were pure *aurea*. No green leaf was seen among them. I planted 180 individuals, 20 of which stayed in the condition of rosettes, whereas most of the others flowered in August. There was a high degree of fluctuating variability in their development. Some were weak and died early, others were strong and flowered richly on the main spike and a number of side branches. All intermediate degrees were seen. But the color was golden without exception and remained so as long as the weather was bright and sunny. In July and August they slowly assumed a pale green color, evidently under the influence of the diminishing number of sunny days, the season being very rainy this year. In this respect they behaved like so many horticultural *aurea* varieties. The whole beds were uniform, apart from the size of the flowers. There were 73 percent of specimens with small flowers, of the size of those of *Oe. biennis* or smaller (petals about

1.5 cm) and 27 percent large-flowered ones (petals 3.5 cm). This points to a Mendelian proportion with the small petals as the dominant character and justifies the expectation that the large-flowered will constitute a constant race. This would be one of the most showy among all the mutants of the *Oenotheras*, apart from its origin from crossed seed. But since neither of the parent species has golden foliage it is evident that this new character must be due to a mutation. In this connection it should here be remembered that it arose only in the third generation, and in eleven specimens.

CROSSES OF *OE. SUAVEOLENS* WITH *OE. COCKERELLI*

In order to control some of the features of the previous cross I repeated it, replacing *Oe. Hookeri* by *Oe. Cockerelli*, a species which is isogamic like the other, but is not known to produce splitting hybrids.

The combination *Oe. Cockerelli* × *Oe. suaveolens* gave a uniform progeny like the corresponding one, described above. It consisted mostly of weak, yellow plants. No second generation has been cultivated.

The combination *Oe. suaveolens* × *Cockerelli* gave in the first generation hybrids of an intermediate type and some aberrant plants with almost linear leaves. They appeared in larger numbers than in the corresponding cross of *Oe. Hookeri*. I made the cross more than once, on one plant in 1913 and on two in 1915. The first gave 126 offspring with 9 percent of linear-leaved specimens, the others gave 14 and 23 percent of this type among 70 and 170 plants. The linear-leaved specimens were all exactly of the same type, with gray hairy foliage and thickly conical flower buds. Moreover their progeny was also uniform. I cultivated 60 specimens of the second and 63 of the third generation, but did not find the least deviation.

The typical hybrids of both crosses resembled one another fully. I had a second and a third generation in 1915 and 1916. The second was uniform, without narrow-leaved plants, but with one *lutescens* among 32 specimens. Two of the typical ones were self-fertilized and yielded each in 1913 a progeny of 70 flowering plants, among which no linear-leaved ones were seen. But 9 and 4 *lutescens* were counted. In order to make wholly sure that no linear-leaved forms are produced by self-fertilized seeds of the hybrids, I repeated the first generation in 1915, self-fertilized five typical specimens and sowed their seeds in 1916. The cultures embraced 40-100, together 382 plants, but only one specimen of the linear-leaved type appeared. It was a weak and low individual, but resembled in all respects the progeny of the linear-leaved individuals of

the first generation. The cultures embraced 28 specimens of *lutescens* and four of the type described as mut. *jaculatrix* (DE VRIES 1916 a).

Thus we see, that our experiments coincide with the cross between *Oe. suaveolens* and *Oe. Hookeri* in regard to the appearance of types which are more or less evidently due to mutations of the former species. But the splitting into two almost equal groups, which is so characteristic of the crosses of *Oe. Hookeri*, is absent here, even as it is absent in all the other crosses of *Oe. Cockerelli*.

SUMMARY

1. *Oenothera Hookeri* T. and G. produces a splitting *laeta* and a uniform *velutina* in its crosses with *Oe. grandiflora*, exactly as it does in those with *Oe. Lamarckiana*.

2. This production of twin hybrids in the first generation is due to the mass mutation of *Oe. grandiflora* into *Oe. mut. ochracea*. The typical gametes produce the *velutina*, whereas the mutated ones give rise to the *laeta*.

3. The percentage figures for these splittings were 41 for *velutina* among the seeds from the cross and 40 for *velutina* in the offspring of self-fertilized *laeta*. The splitting is, in the latter case, unilateral, since there are no empty seeds and since the *laeta* has the same hereditary qualities in its male and its female sexual cells. This is shown by its crosses with *velutina* giving the same splitting (46 percent *velutina*).

4. *Oe. Lamarckiana* \times *Oe. Hookeri* and the reciprocal cross, which produce the twins *laeta* and *velutina*, and whose *laeta* also split into *laeta* and *velutina* in their succeeding generations have no constant *laeta* in their pedigree.

5. *Oe. franciscana* Bartlett, a new species from California, closely related to *Oe. Hookeri*, repeats in its crosses with *Oe. grandiflora* and *Oe. Lamarckiana* the splitting phenomena described for the crosses of *Oe. Hookeri*. Here also the *laeta* repeat the splitting after self-fertilization, whereas the offspring of the *velutina* is constant.

6. There is an almost complete analogy between the twins of *Oe. Lamarckiana* and those of *Oe. grandiflora* and this points to a mass mutation into a *velutina*-producing mutant for *Oe. Lamarckiana*.

7. The same analogy is found for the corresponding crosses of *Oe. Lamarckiana* mut. *velutina* (*Oe. mut. blandina*).

8. The mass mutation observed for *Oe. grandiflora* and assumed for *Oe. Lamarckiana* must lead to a production of their twins in equal numbers and does not explain the splitting of the *laeta* after self-fertilization.

The experimental results give often about equal numbers for the two types, but still more often a deviation from this proportion is observed, which almost always consists in an excess of *velutina*. This shows the splitting capacity of the *laeta* gametes to be present already in the original species.

9. The fecundation of *Oe. Hookeri* by *Oe. biennis* gives uniform hybrids of a type called *rubiennis*, which, in their progeny, split off constant hybrids of the type of *Hookeri* and repeat this in the succeeding generations. No constant specimens of *rubiennis* have been found. The heterogamic condition of *Oe. biennis* seems to afford a sufficient explanation for this unilateral splitting.

10. If the uniform and constant hybrid races *Oe. biennis* \times *syrticola* and *Oe. syrticola* \times *biennis* are fecundated by *Oe. Hookeri* or another species, the same type results as from the corresponding binary crosses. This shows that the paternal hereditary qualities are not transferred to or handed down by the maternal side in these crosses.

11. *Oenothera suaveolens* \times *Oe. Hookeri* behaves just like *Oe. Hookeri* \times *Oe. biennis*. Its first generation consists uniformly of *rubiennis*, but this splits off, in the succeeding generations, specimens of the type of *Hookeri*, which are constant in their progeny. Besides these, however, a noticeable number of different mutants appear.

12. Corresponding mutants were observed among the progeny of the cross *Oe. suaveolens* \times *Oe. Cockerelli*, the hybrids of which do not show any regular splitting.

13. In their crosses with the pollen of *Oe. biennis*, the new types, *Oe. franciscana* and *Oe. Lamarckiana* mut. *velutina*, repeat the splitting produced by *Oe. Hookeri*. The first generation is uniform and shows a predominance of the characters of the pollen parent. The second splits into this same type and another, which is almost like the second grandparent. In the numerical proportions these latter prevail, about 20-30 percent being *rubiennis* and about 70-80 percent belonging to the other type.

14. The crosses of *Oe. grandiflora* and *Oe. Hookeri*, both of which are large-flowered species, produce hybrids which split off small-flowered specimens. The size of these flowers is about that of *Oe. biennis* L. The large flowers dominate, the small are found in a proportion (27 percent) which corresponds to the formula of MENDEL for monohybrids.

15. In the hybrids between the large-flowered *Oe. suaveolens* and *Oe. Hookeri*, a splitting as to the size of the flowers was also observed, but

here the small size, corresponding to that of *Oe. biennis*, was dominant over the large flowers of both the grandparents.

16. From this same cross a new and beautiful mutant arose, being a pure *aurea*, with bright golden leaves. It appeared in the third generation in eleven specimens, without intergrades or transitions, and yielded a uniformly golden second generation.

17. Summarizing all these facts we find that splitting *laeta* and splitting *rubiennis* are produced by three large-flowered races, and by these only, as yet. These races are two Californian species: *Oe. Hookeri* T. & G. and *Oe. franciscana* Bartlett, and a mutant which originated in my garden, *Oe. Lamarckiana* mut. *velutina* (syn: *Oe. mut. blandina*). In the large number of other cases the hybrids of the *Oenotheras*, whether twins or monotypic, are constant in their progeny, apart from the rare cases of Mendelian splitting. These *laeta* and *rubiennis* always split into two types, one of which repeats their marks, whereas the second resembles the other grandparent. No constant specimens of *laeta* or *rubiennis* have been found among them.

LITERATURE CITED

- BARTLETT, H. H., 1914 Systematic studies on *Oenothera* IV. *Oe. franciscana* and *Oe. venusta* spp. nov. *Rhodora* **16**: 33-37.
- DAVIS, B. M., 1916 Hybrids of *Oenothera biennis* and *Oenothera franciscana* in the first and second generations. *Genetics* **1**: 197-251.
- GOLDSCHMIDT, R., 1912 Die Merogonie der *Oenothera*-Bastarde. *Archiv f. Zellforschung* **9**: 331-343.
- RENNER, O., 1913 Ueber die angebliche Merogonie der *Oenothera* Bastarde. *Ber. d. deutsch. bot. Ges.* **31**: 334-335.
- DE VRIES, H., 1913 Gruppenweise Artbildung. pp. xiv + 353. Berlin: Gebr. Borntraeger.
- 1915 *Oenothera gigas nanella*, a Mendelian mutant. *Bot. Gaz.* **40**: 337-345.
- 1916 a Die endemischen Pflanzen von Ceylon und die mutierenden *Oenotheren*. *Biol. Centralbl.* **36**: 1-11.
- 1916 b Gute, harte und leere Samen von *Oenothera*. *Zeitschr. f. indukt. Abst. u. Vererb.* **16**: 239-292.
- 1917 a *Oenothera Lamarckiana* mut. *velutina*. *Bot. Gaz.* **63**: 1-25, plate 1.
- 1917 b Kreuzungen mit *Oenothera Lamarckiana* mut. *velutina*. *Zeitschr. f. indukt. Abst. u. Vererb.* **19**: 1-38.
- 1918 a Mutations of *Oenothera suaveolens* Desf. *Genetics* **3**: 1-26, 4 figs.
- 1918 b Mass mutations and twin hybrids of *Oenothera grandiflora* Ait. *Bot. Gaz.* **65**: 377-422.