

AA (or aa) individuals $= 2s(o_n) + s(p_n) + 1/2s(r_n)$,

Aa (or aA) individuals $= 2s(q_n) + s(1/2p_n) + 1/2s(r_n)$.

The first of the above expressions multiplied by 2 gives the total homozygotes, and the second multiplied by 2 gives the total heterozygotes.

RAYMOND PEARL

PARALLEL MUTATIONS IN *OENOTHERA BIENNIS* L.

IN the summer of 1912 I cultivated pure strains of *O. biennis* L. and of the *O. biennis cruciata* de Vr. of our Dutch dunes, as well as of their hybrids, made with the purpose of studying the behavior of the *cruciata*-character in crosses. In one of these cultures I unexpectedly obtained two mutants, which because of their similarity to corresponding variants derived from *O. Lamarckiana* have been called *O. biennis nanella* and *O. biennis semi-gigas*. The first mutant, *O. biennis nanella*, occurred in the second generation of the cross *O. biennis* \times *O. biennis cruciata* and differed from *O. biennis* in all those points which separate *O. Lamarckiana nanella* from *O. Lamarckiana*. The other variant, *O. biennis semi-gigas*, appeared in the second generation of the reciprocal cross, *O. biennis cruciata* \times *O. biennis*, suggesting immediately by its much more vigorous habit and especially by the larger size of its buds and flowers the differences between *O. Lamarckiana* and *O. gigas*. A count of its diploid number of chromosomes proved it to deserve the name *semi-gigas*, 21 chromosomes being shown by nuclear plate-stages in the meristematic tissue of young buds. From these facts, showing that *O. biennis* is in a mutating condition, I drew the conclusion that the phenomenon of mutation in the genus *Oenothera* is older than the species *O. Lamarckiana*—*O. biennis* generally being considered to be an older species than *O. Lamarckiana*—and further, that the mutations in this group can not be the result of hybridization, as was assumed by some authors at that time—nobody doubting of the purity and constancy of *O. biennis*. As a matter of fact, both of my mutants have been derived from crosses between *O. biennis* and *O. biennis cruciata*. But I laid special emphasis on the fact that *O. biennis* and *O. biennis cruciata* have exactly the same germinal constitution except for the factors that determine the shape of the petals, *O. biennis cruciata* being prob-

ably a mutant from *O. biennis* itself. Therefore, hybrids between these two forms can be looked upon as pure *O. biennis* except for floral characters.

With this conception Bradley Moore Davis does not agree.¹ He thinks that the *O. biennis* and *O. biennis cruciata* of our dunes are not so closely related types, that a cross between them can be treated "as though it were the combination of forms within the same species which have similar germinal constitutions." He says:

It should be made clear that the form "*O. biennis cruciata*" is recognized in the more recent taxonomic treatments as a true species sharply distinguished from types of *biennis* by its floral characters. . . . *O. cruciata* is found wild in certain regions of New England and New York and is consequently a native American species. . . . Whatever may have been the origin of *O. cruciata* or its possible relationship to *O. biennis*, a cross between these types must certainly be regarded as a cross between two very distinct evolutionary lines and its product a hybrid in which marked modifications of germinal constitution are to be expected.

From Davis's point of view I "really made a cross between two rather closely related species" and obtained in the second generation "two marked variants due to some germinal modifications as the result of the cross." In so far as my observations bear upon the problem of mutation Davis's interpretation is exactly the reverse of mine. To him they further illustrate the same phenomenon which he is obtaining through his "hybrids of *biennis* and *grandiflora*, namely, that behavior by which these hybrids in the F_2 generation throw off variants that in taxonomic practise would be considered new species readily distinguished from the parents of the cross and from the F_1 hybrid."

It will be shown in the following lines that the objections made by Davis are not sufficiently justified. My argument consists of two points.

In the first place, Davis is mistaken as to the nature of the *O. biennis cruciata* de Vr. of our dunes. This strain is in reality quite another type than the different forms of the American *O. cruciata* Nutt., called by some authors *O. biennis cruciata*. With this species it has in common only the character of the narrow

¹ Bradley Moore Davis, "Mutation in *Oenothera biennis* L.?" THE AMERICAN NATURALIST, Vol. XLVII, 1913, pp. 116-121; "Genetical Studies on *Oenothera*," IV, THE AMERICAN NATURALIST, Vol. XLVII, 1913, pp. 546-571.

petals, all other features of the stem, foliage, flowerspikes and fruits being exactly those of the Dutch *O. biennis* L. It must certainly be looked upon as a mutation from the *O. biennis* L. of our sand dunes. Until now it has only been found a couple of times in single individuals in the midst of the ordinary *O. biennis*, the first time in 1900 by Dr. Ernst de Vries in the dunes in the neighborhood of Santpoort, Holland, in one individual—and from this one specimen all the subsequent generations of *O. biennis cruciata* in the cultures grown by de Vries and by myself have been derived. Besides this, our *O. biennis* and *O. biennis cruciata* are so similar to one another except for floral structure that plants of both types can not be separated before the flowers open. Therefore we have the right to assume that the crossing of these two forms is concerned alone with the floral characters and that with respect to all other characters parents as well as hybrids are mere *biennis*. Therefore the two variants which arose in my cultures from crosses between *O. biennis* and *O. biennis cruciata* obviously prove the faculty of mutation in *O. biennis*.

In the second place I have found now that it is not necessary to cross *O. biennis* with *O. biennis cruciata* in order to obtain the above named mutants, as Davis seems to believe. Already in his new book Professor de Vries figures a dwarf derived from *O. biennis cruciata* grown in pure line. Shortly afterwards I myself obtained six mutants from the *O. biennis* of our sand dunes grown also in pure line. A few details about these cultures of last year may be given here. In all they counted 920 individuals, 430 of which belonged to the third and 490 to the fourth generation of a pure line, the point of departure for which had been one individual brought into the experimental garden in the rosette stage from the dunes near Wyk aan Zee in the beginning of 1905 and self-fertilized in the same year. The six mutants which appeared in these pure cultures of *O. biennis* were the following. First a dwarf, then a *biennis semi-gigas* having 21 chromosomes and finally four individuals of the *O. biennis sulfurea*, a pale-flowered form of *O. biennis*, which had been found already several times in our dunes in the midst of the ordinary *biennis*, but was not with certainty known to be a mutant from the latter form until now. The two first named mutants and one *sulfurea* appeared in the third generation of our pure line, the *nanella* and the *semi-gigas* coming from the same mother. The three remaining *sulfurea* individuals appeared in the fourth generation, all

descending from the same motherplant. Of these mutants the *nanella* and *semi-gigas* are especially valuable because similar forms have been produced by *O. Lamarckiana*. It will be seen that the *biennis*-dwarfs seem to be somewhat rarer than the dwarfs of *Lamarckiana*. Whilst for the latter the mutation coefficient is about 1 per cent., our *O. biennis nanella* appeared as the only dwarf among 920 individuals. The above cited *O. biennis cruciata nanella* was the only dwarfed individual in a culture of 500. And the dwarf which I got in 1911 was the only one among about 600 plants. In this connection I wish to recall the conclusion reached by de Vries that in *O. Lamarckiana* the pangen for tall stature must be assumed to be present in the labile condition on both sides, in *O. biennis*, however, only in the male sexual type, whilst in the female sexual type active alta-pangens have to be supposed. The way from *biennis* to *biennis nanella* might therefore possibly be somewhat longer than the one from *Lamarckiana* to *Lamarckiana nanella*. The *biennis semi-gigas* which appeared in the last summer corresponded in all points exactly with the mutant of 1911. Moreover a count of the chromosomes, as shown by nuclear plate-stages in the meristematic tissue of young buds, determined them to be 21 in number. Even as the specimen of this type, that appeared in 1911, and as the *semi-gigas* mutants produced by *O. Lamarckiana*, the plant of last year proved to be almost absolutely sterile.

In his second above-mentioned paper Davis says about the *O. biennis* of our dunes: "No species of *Ænothera* is perhaps so free from suspicion as to its gametic purity. If Stomps can obtain mutations from tested material of the Dutch *biennis* grown in pure lines he will have the basis of a strong argument. . . ." Fortunately the experiment asked for by Davis, has been made in the same year as his criticism. The Dutch *biennis* L., cultivated in pure line, has produced a dwarf, a *semi-gigas* and some *sulfurea*-individuals, proving its mutability beyond all doubt. I therefore trust that the conclusions arrived at in my first paper, concerning this mutability and its consequences, may now be accepted as thoroughly valid.

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IN a recent review¹ of Stomps's studies on *Oenothera biennis* L.² from the sand dunes of Holland I protested against his designating as mutants a *nanella* type and a *semi-gigas* type which were obtained in the second generation of crosses between *Oenothera biennis* Linnæus and its variety *O. biennis cruciata* de Vries. The criticism was presented on the general ground that however close the possible relationships between the two parent forms, they nevertheless constituted lines so far apart as to render unsafe a conclusion that marked variants obtained from their crossing are mutants in the sense of de Vries and Stomps. Such variants, it seemed to me, might have been the result of hybridism between two lines sufficiently divergent to upset the similarity of germinal constitution shown in their vegetative morphology, for the species *biennis* and its variety *cruciata* are said to differ only in their flower structure.

In that review I incorrectly associated *O. biennis cruciata* de Vries with *O. cruciata* Nutt., an American species entirely distinct from the variety *cruciata* of de Vries, which has been found only once (in the year 1900) on the sand dunes of Holland among plants of *O. biennis*. I greatly regret my confusion of these two types, since I was led in my criticism to regard Stomps's crosses between *biennis* and *biennis cruciata* as though they were crosses between two distinct although possibly closely related species. In this I was clearly mistaken, since all of the evidence short of experimental proof, which Stomps may yet obtain, indicates that *biennis cruciata* de Vries is a variety of *biennis* L. and arose as a mutation on the sand dunes of Holland. The crosses of Stomps are, therefore, to be regarded as between a species and its mutant variety. I trust that the mutationists will accept this acknowledgment of an error.

There is, I believe, a body of naturalists for whom the value of evidence for mutation rests fundamentally upon the unquestioned purity of the parent stock, and to them any cross, no matter how close, is open to criticism. Stomps has justified his first conclusions by obtaining in later studies the same mutants *biennis nanella* and *biennis semi-gigas* from lines of the pure species *O. biennis* Linnæus. Had he waited for these later results before

¹ Davis, B. M., "Mutations in *Oenothera biennis* L.?" AMERICAN NATURALIST, Vol. XLVII, p. 116, 1913.

² Stomps, T. J., "Mutation bei *Oenothera biennis* L.," Biol. Centralb., Vol. XXXII, p. 521, 1912.

publishing on the first there could have been no objections to his main contention that *O. biennis* from the sand dunes of Holland is capable of giving rise to true mutants.

Stomps is continuing his studies on this same Dutch *biennis* with the view of determining its possible powers of mutation, and it is a pleasure to review his second paper³ which presents some extremely interesting data, a paper in which no important criticism can be based on the source and character of the material employed. No wild species of evening primrose has been so long under experimental and field observation or is better known to the workers with *cenotheras* than this plant. The species has proved uniform in culture to a remarkable degree and it would be difficult to find a type of *Cenothera* so free from suspicion of gametic purity. The species appears to have been in Holland since pre-Linnæan days and is therefore very old. As material for experimental studies on mutation the Dutch *biennis* seems to the writer the best of all the *cenotheras* so far brought into the experimental garden.

The starting point of Stomps's cultures of *Cenothera biennis* was a plant transplanted from the sand dunes in 1905. From seed of this plant, self-pollinated, a second generation was grown in 1910, three selfed plants of which gave the seed for a third generation of 430 individuals, and a fourth generation of 490 plants was grown from two selfed plants of the third generation. Thus in all 930 individuals were observed in the third and fourth generations from the plant that gave rise to these pure lines. It is true that these lines have not been under selection for many generations, but, considering the stability of the species and its habit of close pollination, it is very improbable that the source of the cultures should have been a plant not representative of the type. Furthermore, Stomps presumably will continue indefinitely the lines now established and thus determine through later generations whether their mutating habits remain constant.

Among the 430 plants of the third generation there appeared 1 *biennis nanella*, 1 *biennis semi-gigas* and 1 individual of *biennis sulfurea*; the first two came from the same mother plant. Among the 490 plants of the fourth generation appeared 3 individuals of *biennis sulfurea*, all from the same selfed mother. The variety *sulfurea* differs from the species *biennis* in having flowers of a

³ Stomps, T. J., "Parallele Mutationen bei *Cenothera biennis* L.," *Ber. deut. bot. Gesell.*, Vol. XXXII, p. 179, 1914.

lighter yellow, and is reported by de Vries to be not uncommon in the wild state mixed with the species proper. *Sulfurea* has been held systematically to be a variety of *biennis* but this is the first time that it has appeared in the experimental garden as a derivative of that species. Thus out of a total of 920 plants there were 4 individuals of the color variety *sulfurea*, 1 *nanella* and 1 *semi-gigas*, in all 6 mutants, a showing that may well gratify Stomps.

The mutant *biennis nanella* differed from typical *biennis* in much the same way that *Lamarckiana nanella* differs from *Lamarckiana* and like the latter dwarf showed evidence of a bacterial infection. Certain selfed flowers set no seed because of diseased stigmas. Other flowers pollinated from pure *biennis* set good fruit. The ratio of the appearance of *biennis nanella* is much lower than the mutation coefficient of one per cent. which de Vries has reported for *Lamarckiana nanella*. It should also be remembered that de Vries⁴ obtained a *cruciata nanella* in a culture of 500 plants from *O. biennis cruciata*.

The mutant *biennis semi-gigas* in comparison with typical *biennis* showed a stronger habit, broader leaves, thicker buds, larger flowers, supernumerary stigma lobes, and the presence of 4-cornered pollen grains. Counts of the chromosomes in meristematic tissue determined the number to be 21. Therefore in this plant, as in the *biennis semi-gigas* obtained by Stomps from the cross *cruciata* \times *biennis*, there is clear cytological evidence that one of the gametes which formed the zygote contained 14 chromosomes, *i. e.*, double the number characteristic of the gametes of *Oenothera*. This is another case of triploid mutants in *Oenothera* to be added to the list of Stomps and Miss Lutz. The plant was self sterile, but set fruit when pollinated by *biennis*, although the yield of seed was very poor.

Stomps is justified in calling attention to the agreement of his second *biennis nanella* with the plant derived in 1911 from the cross *biennis* \times *cruciata*, and of the agreement of his second *biennis semi-gigas* with the plant from the cross *cruciata* \times *biennis*. It is to be hoped that he will next obtain the *cruciata* variety as a direct mutant from the Dutch *biennis* and thus establish its relationship and origin beyond all possible doubt.

Stomps has before him the opportunity of making through the study of *Oenothera biennis* very important contributions to our

⁴ See "Gruppenweise Artbildung," p. 299 and Fig. 108.

knowledge of the frequency of mutations and their importance in organic evolution. That retrogressive mutations take place is not likely to be seriously doubted by any one who has followed the experimental work of recent years both botanical and zoological. The loss of characters through germinal modification, even in what seem to be "pure lines," appears to be not uncommon.

Most of all is desired information on the possibilities, frequency and character of progressive mutations. Can the mutation theory satisfactorily explain progressive advances in organic evolution or must amphimixis chiefly carry that responsibility? Mutants of the tetraploid *gigas*-like type would appear to be progressive, and we can see the reason in their doubled chromosome count which gives larger nuclei, larger cells and modified tissues. *Gigas*-like forms are however very rare and in *O. Lamarckiana* *gigas* the fertility is relatively low. More common are the triploid *semi-gigas* forms, but these seem to be sterile or almost sterile when selfed, and the work of Geerts indicates that the triploid number in *Ænothera* returns to the normal through the elimination of supernumerary chromosomes. Very interesting is the recent paper of Gates and Thomas⁵ which offers evidence that *lata*-like characters are associated with the presence of a single additional chromosome.

And what of the series of forms which differ from the *Ænothera* parent types with as yet no evidence of peculiarities in their chromosome count, *brevistylis*, *lavifolia*, *rubrinervis*, *obovata*, *scintillans*, etc. Will forms similar to these and perhaps others in addition be represented in a series of derivatives from *Ænothera biennis*? The mutants *biennis nanella* and *biennis sulfurea* belong to this group and have already been obtained by Stomps. One may almost envy him his opportunity for an intensive study of this species.

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⁵ Gates, R. R., and Thomas, N., "A Cytological Study of *Ænothera* mut. *lata* and *Æ.* mut. *semilata* in Relation to Mutation," *Quart. Jour. Mic. Sci.*, Vol. LIX, p. 523, 1914.