THE SEGREGATION OF *OENOTHERA BREVISTYLIS* FROM CROSSES WITH *OENOTHERA LAMARCKIANA*¹

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INTRODUCTION

Genetical work upon the *Oenotheras* has developed a mass of data which has not as yet been interpreted in simple terms. There is the phenomenon called by DE VRIES and his followers "mutation," most strikingly illustrated in the appearance of marked variants from supposedly pure races or species when inbred. There are the peculiar patroclinous and matroclinous conditions presented in some F₁ generations and the subsequent reappearance of the parental characters in certain inter- and back-crosses. There is the production of two, three or even four types as the immediate result of certain crosses giving the so-called twin, triple and quadruple hybrids, where one would expect a uniform generation if the parental forms were homozygous. And there are a few cases reported in which the inheritance appears to follow a fairly simple Mendelian system which is at least clear as far as the fundamental principle of segregation is concerned although the ratios may be somewhat irregular.

¹ Genetical studies on *Oenothera* IX.

The writer has in recent years (DAVIS 1915 b, 1916 a, b, 1917) contended that these peculiarities of *Oenothera* genetics are due at least in large part if not wholly to a wide-spread genetical impurity general to the species and races of this group of plants and also to the high degrees

FIGURE 1.—Rosette of *Lamarckiana*, leaves mostly acute-pointed. Contrast with figure 2, *brevistylis*.

of gametic and zygotic sterility which makes it seem probable that our cultures generally represent imperfectly the behavior which would be expected if in supposedly complex material all of the gametes and zygotes were viable. Large proportions of the seeds of *Oenothera* through delayed germination unquestionably have not recorded their potential

products in the cultures of past years and only recently have methods been developed (DE VRIES 1915, DAVIS 1915 a) which make certain the germination of all or almost all of the viable seeds and permit of an examination of the residue of sterile structures. With the development of more perfect technique in the germination of seeds and in the establishment of cultures we may in the future hope for more exact data.

The hypothesis which largely forms the basis of the writer's present

FIGURE 2.—Rosette of *brevistylis*, leaves obtusely pointed. Contrast with figure 1, *Lamarckiana*.

attitude (DAVIS 1915 b) postulates the existence in the *Oenotheras* of numerous impure or hybrid species, heterozygous in their germinal constitution, but which breed fairly true because for the most part only those gametes and zygotes are viable which may reproduce the parent type. Thus extensive gametic and zygotic sterility may be conceived to hold a hybrid line to a habit of relatively true breeding even though its germinal constitution remain heterozygous. The elimination through sterility of all gametes and zygotes excepting those which may carry

forward the heterozygous constitution of a hybrid may give an appearance of genetic purity which is the reverse of the facts. True breeding is not the final test of a pure species. Animals and plants must be shown to breed true because their germinal constitution permits the reduction divisions to give only identical products, except when sex differentiation is present, and the gametes must therefore be uniform for genetical factors that are not associated with sex. As the writer has emphasized in former papers no material of *Oenothera* has as yet passed the tests (DAVIS 1915 b) which justify our acceptance of it as representative of a pure species.

I do not care at this time to discuss in detail the bearing of this hypothesis of the impure species in its relation to the problems of *Oenothera* genetics but it may be remarked (1) that by the hypothesis most "mutations" may prove to be more readily interpreted as products of diverse gametic combinations from heterozygous material and that their presence in greater or less proportions depends upon their nature and is subject to the effects of sterility, and (2) that twin, triple and quadruple hybrids indicate that one or both of the parents cannot be homozygous since they are regularly producing different types of gametes which in these particular crosses prove to be fertile with one another.

Of the *Oenothera* material available for observations on possible Mendelian inheritance there is, as so far described, perhaps none more satisfactory for the characters concerned or more simple in its genetical nature than *Oe. brevistylis* crossed with *Oe. Lamarckiana*. The behavior of these hybrids gave the first suggestion of Mendelian system in *Oenothera* genetics and the only published studies upon the material have been those of Professor DE VRIES (1901-'03, Vol. 2, pp. 157, 159, 429-442; 1913, pp. 188-190) who made crosses of *brevistylis* not only with *Lamarckiana*, but with certain "mutants" from *Lamarckiana* and with some other species.

Other studies on *Oenothera* have described behavior apparently Mendelian in character. GATES (1915 a, p. 226) through a series of papers reached the conclusion that the peculiar pigmentation of *Oenothera rubricalyx* is dominant in crosses and holds that *rubrinervis* segregates from heterozygous *rubricalyx* in proportions approximating a 1:3 ratio. To his interpretations SHULL (1914) takes exception but GATES (1915 b) in a later paper reaffirms his position. DE VRIES (1915) reports the isolation of a heterozygous line of *gigas* that gave in one generation about 18 percent dwarfs, about 25 percent pure *gigas*, and about 57 percent of a *gigas*-like form, similar to the parent, which when selfed

proved itself to be heterozygous by again throwing about 21 percent of dwarfs.

DE VRIES reported the characters of *brevistylis* to be recessive in the F_1 generations of its crosses and then to appear in the F_2 generations in proportions fairly close to 25 percent of the progeny, which would be the expected Mendelian ratio for a simple monohybrid situation. My observations support the conclusions of DE VRIES. I have, however, data on a number of inter- and back-crosses not made by DE VRIES, and the cultures of the past two years have been grown from seed forced to complete germination. Consequently we have now before us considerably more data and that probably more reliable because of the methods of seed germination employed. There is, nevertheless a range of variation from expectations in the ratios of *brevistylis* segregates, the reasons for which are not at present clear. This matter will be taken up in the discussion.

METHODS OF CULTURE

The cultures of 1916 and 1917 were from seeds handled in the following manner. The entire contents of seed capsules were placed in vials half-filled with water and allowed to soak for 24–48 hours. The vials were then placed uncorked in a metal case made of iron pipe the cover of which can be fastened air tight by bolts. There is a pressure gauge attached to the cover and at the bottom of the case is an inlet tube such as is fitted into an automobile tire. Air was then forced into the case with an automobile pump until the pressure registered 100 pounds. The seeds in the water were left in the case under this air pressure for 24–36 hours. The contents of the vials were then poured over pads of filter paper in Petri dishes and the seeds evenly distributed. In 1916 the Petri dishes were placed in an incubator at temperatures from 28–31° C, but later experiments showed that germination in this material occurs quickly and completely when the Petri dishes are kept in a hot-house and I am inclined to think that some variations in temperature may favor rapid germination. It is surprising to see how quick may be the response in warm sunny weather when the temperature of a hot-house runs up high in the day time.

As rapidly as the seeds germinated they were removed to other Petri dishes and laid on wet pads of filter paper until the seedlings had so developed that they could be readily handled with the forceps and set into earth. The germinated seeds were counted and the seedlings were planted in rows in square earthen seed pans and any later losses through

death were thus readily noted. From the seed pans the young plants were transplanted to small pots. Seed-sterile soil was used in all cultures while in the hothouse. Cultures started in January and February produced strong rosettes by May which when set in the garden developed mature plants by early summer.

When, after 5-6 weeks, germination of the seeds seemed complete the

FIGURE 3.—Inflorescence of *Lamarckiana* viewed from above, showing narrower bud cones and attenuate sepal tips. Contrast with figure 4, *brevistylis*.

residue of ungerminated structures were tested for contents by pinching each seed with strong forceps. Most of the structures were found to be entirely empty, but a few contained small amounts of material readily squeezed out by the pressure of the forceps. Very rarely were any ungerminated seeds found, as shown by the presence of embryos. This residue of sterile structures arranged, dried, and fastened by shellac or glue on a piece of filter paper presented a record which together with

the number of germinated seeds enabled me to determine very exactly the percentage of seed fertility.

It is interesting to note in the tables how much higher in general is the percentage of germination obtained by this method of culture over the

FIGURE 4.—Inflorescence of *brevistylis* viewed from above, showing stouter bud cones and shorter sepal tips. Contrast with figure 3, *Lamarckiana*.

old practice of sowing seeds in the earth. The satisfaction of handling cultures with complete germination, thus giving accurate data on the percentage of seed sterility, is well worth the trouble involved and indeed is quite necessary where accuracy of results, so essential to Mendelian studies, are to be obtained in the group of the *Oenotheras*.

A COMPARISON OF THE PARENT SPECIES, *OENOTHERA LAMARCKIANA* AND
OE. BREVISTYLIS

Although *Oenothera brevistylis* has never been observed in the lines of *Lamarckiana* cultivated by DE VRIES or derived from his material, its origin from this plant seems quite certain. It was reported by SCHOUTEN (1908) in a culture of *Lamarckiana* from commercial seeds, 6 plants of *brevistylis* among 502 *Lamarckiana* with 7 *lata*, 1 *nanella*, 3 *gigas* and 1

FIGURE 5.—Buds of *Lamarckiana*, *brevistylis*, and of the F_1 hybrids. Compare the sepal tips, the relative width of bud cones and the relative width of bracts. Note the contrasts between *Lamarckiana* and *brevistylis* and the high degree of dominance of *Lamarckiana* characters shown in the hybrids.

rubrinervis (ref. GATES 1915 a, p. 90). *Oenothera brevistylis* was discovered by DE VRIES in 1886 in the abandoned field at Hilversum growing among the plants of *Lamarckiana* and is known to have maintained itself in this locality for seventeen years. Although *brevistylis* sets very little selfed seed the fact that it maintains itself under suitable conditions is not remarkable since the plant pollinates *Lamarckiana* readily and the type segregates out perfectly in the F_2 generations from both of the reciprocal F_1 hybrids, from the double reciprocals, and from the back crosses of the F_1 hybrids with *brevistylis*. Furthermore, like a re-

cessive, which it is, *brevistylis* breeds perfectly true when selfed, although very small amounts of seed are produced even on favorable plants.

Oenothera brevistylis has been thoroughly described by POHL (1895) and DE VRIES (1903, p. 429). It differs from *Oe. Lamarckiana* in a number of characters so closely associated that so far as I know they have always been observed to be inherited as a group. These characters are contrasted in the following table.

<i>Oenothera Lamarckiana</i>	<i>Oenothera brevistylis</i>
Mature rosettes. Leaves acute-pointed (fig. 1).	Mature rosettes. Leaves obtuse-pointed (fig. 2).
Bracts. Narrower and acute-pointed (figs. 3 and 5).	Bracts. Broader and less pointed (figs. 4 and 5).
Buds. Longer 8—9 cm, their cones narrower, and the sepal tips attenuate, 7—8 mm long (figs. 3 and 5).	Buds. Shorter 5.5—6 cm, their cones more stout, and the sepal tips short, 1—2 mm long (figs. 4 and 5).
Style. Long, projecting 3—6 mm above the tips of the anthers (fig. 6).	Style. Short, generally terminating near the top of the calyx tube (fig. 6).
Stigma lobes. Well developed, 5—6 mm long (fig. 6).	Stigma lobes. Short and frequently rudimentary (fig. 6).
Ovary. Inferior, the ovules lying well below the insertion of the calyx tube (fig. 6).	Ovary. Partly superior, extending slightly above insertion of the calyx tube (fig. 6).
Capsules. Stout, 18—25 mm long, ripening from 100-350 seeds.	Capsules. Slender, scarcely longer than the ovaries, 9—11 mm, ripening no seeds or very few.

Plants of *Oenothera brevistylis* may be selected in the rosette stage by the practiced eye, but, as DE VRIES has pointed out, they do not stand out conspicuously among plants of *Lamarckiana* until the inflorescence begins to develop, when the broader and less pointed bracts and the very short sepal tips present sharp and clear differentiating characters. The absence of large capsules further distinguishes an older plant of *brevistylis* and the much longer flowering season is probably the result of its almost complete failure to ripen seed, thus conserving to the plant energy for a more extended period of flowering and vegetative growth.

Two races of *Oenothera Lamarckiana* have been employed in the crosses with *brevistylis*. *Lamarckiana* S came to me as a cross-bred

race developed by SHULL from nine lines of *Lamarckiana* originally obtained by DE VRIES from the field in Hilversum. I received seeds of *Lamarckiana* S (SHULL's culture 092.2) in 1911 and have carried a selfed line through six generations from that date. *Lamarckiana* M is a line received directly from DE VRIES and grown in my garden since 1910. These two lines are quite indistinguishable in their morphology, such differences in the measurements of their organs being too small to catch the eye, but they differ in their seed production and seed fertility. *Lamarckiana* M gives a yield of 200-350 seeds per capsule and the tested

FIGURE 6.—Ovaries, styles and stigmas of *Lamarckiana*, *brevistylis* and the F_1 hybrid. Compare the relative lengths of the style, the degree of development of the stigma lobes and the extent of the ovarial cavity. Note the contrasts between *Lamarckiana* and *brevistylis* and the high degree of dominance of *Lamarckiana* characters shown in the hybrid.

germination in the last three generations (1915-17) has been respectively 32, 36 and 36 percent. *Lamarckiana* S develops from 100-250 seeds per capsule and the tested germination for the same years was 26, 30 and 31.8 percent. Each germination test was based on the contents of two large-sized capsules.

My material of *brevistylis* came to me through seeds set by an F_1 generation of *Lamarckiana* $\times$ *brevistylis* kindly sent to me by Professor DE VRIES. Although these seeds by the label on the package were

collected in 1907, I obtained germination and a culture of the F_2 generation in 1913. The culture gave the usual clean segregation of *brevistylis* and *Lamarckiana* characteristic of the second generation of this cross. One of the *brevistylis* plants was the parent of the earlier crosses with *Lamarckiana* described in this paper from which other plants of *brevistylis*, segregated in F_2 generations, were obtained for later crosses and for the selfed line of *brevistylis* started in 1916.

THE F_1 RECIPROCAL CROSSES

It is an easy matter to pollinate *Lamarckiana* by *brevistylis* and this cross was early made by DE VRIES who described the characters of the F_1 hybrid. The pollen of *brevistylis* is formed in abundance but as in *Lamarckiana* about 50 percent of the grains are shriveled; the good pollen, however, appears to be as effective as that of *Lamarckiana*. In general it may be said that the characters of *Lamarckiana* are strongly dominant over those of *brevistylis*. In the F_1 hybrid the rosette leaves are acute-pointed, the bracts narrow and acute-pointed, the sepal tips attenuate, the style long with well developed stigma lobes, the ovary inferior, the capsules large and ripening numerous seeds (180-220). I have made this cross employing both *Lamarckiana* S and *Lamarckiana* M as the seed-bearing parent and have found the germination percentage of *Lamarckiana* M $\times$ *brevistylis* (culture 16.14) to be 27.7 percent (two capsules tested with 360 seed-like structures) which is below the tested germination, 32-36 percent, for *Lamarckiana* M.

So similar is the F_1 hybrid of *Lamarckiana* $\times$ *brevistylis* to *Lamarckiana* that neighboring cultures cannot be distinguished excepting when the measurements of strongly contrasted characters are critically compared. Thus the style of the F_1 hybrid may not extend so far above the tips of the anthers as in *Lamarckiana* and occasionally plants are found with some of the flowers presenting stigma lobes midway between the anther tips and the top of the calyx tube. These occasional shorter-styled plants presented other characters somewhat intermediate between *brevistylis* and *Lamarckiana*. Their foliage leaves and bracts were broader than those of *Lamarckiana*, the bud cones were thicker and the sepal tips shorter, but the stigma lobes, while shorter, were not deformed and the yield of seed was good although smaller than that of *Lamarckiana*. The F_2 generations from two of these shorter-styled plants (14.15 y and 14.15 z) presented a clear segregation of *brevistylis* and *Lamarckiana* types (table 1) and I am forced to conclude that the peculiarities of the F_1 parents did not signify genetical differences from the usual F_1

hybrids of *Lamarckiana* $\times$ *brevistylis*. These plants were apparently instances of imperfect dominance where strong dominance is the rule.

The reciprocal cross *brevistylis* $\times$ *Lamarckiana* seems not to have been reported by DE VRIES. It can be made and a small yield of seeds obtained by pollinating a large number of *brevistylis* flowers selecting those with better-developed stigma lobes. Thus after pollinating many flowers of *brevistylis* by *Lamarckiana* S in 1915 I obtained a total of 30 small capsules which altogether yielded only 85 seed-like structures from which 19 seedlings appeared in 1916. Pollinations of *brevistylis* by *Lamarckiana* M made in 1916 gave 58 seed-like structures from 18 capsules and in 1917 (culture 17.59) a total of 24 seedlings, a germination of 41 percent. This was higher than the germination of 27.7 percent shown by the reverse cross *Lamarckiana* M $\times$ *brevistylis* (culture 16.14), but the small numbers of seeds and seedlings obtained in the cross *brevistylis* $\times$ *Lamarckiana* M makes it unsafe to assume that 41 percent is very close to the correct germination proportions.

Plants of the F_1 hybrids *brevistylis* $\times$ *Lamarckiana* are so similar to plants of the reciprocals *Lamarckiana* $\times$ *brevistylis* that I know of no distinguishing differences. As in the reciprocal cross the characters of *Lamarckiana* are strongly dominant in the F_1 generation of *brevistylis* $\times$ *Lamarckiana*.

THE F_2 GENERATION FROM THE F_1 OF *LAMARCKIANA* $\times$ *BREVISTYLIS*

My data on this generation is based on families derived from eleven sister plants in an F_1 generation (culture 14.15) of the cross *Lamarckiana* S $\times$ *brevistylis*. The F_1 parent plants were designated 14.15 *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *y* and *z*. The F_2 families from 14.15 *b*, *c*, and *d* were grown in 1915 from seed sown in earth. The families from 14.15 *e* and *f* were grown by Dr. G. H. SHULL at Cold Spring Harbor, Long Island, in 1915, also from seed sown in earth. The families from 14.15 *g*, *h*, *i*, and *j* were grown in 1916 from seed experimentally germinated as described above under Methods of Culture, the seedlings being set in pans. The F_1 plants 14.15 *y* and *z* were of the type with styles on some of the flowers midway between the tips of the anthers and the top of the calyx tube (see p. 511); from seed of such flowers selfed two families were grown in 1915, the seeds being sown in earth.

All of the F_2 families presented a perfectly sharp segregation of *brevistylis* plants from *Lamarckiana* but with somewhat irregular ratios. The ratios, however, are close enough to the simple Mendelian 1 : 3 to justify the hypothesis that *brevistylis* is a simple recessive from *Lamarck-*

iana and that the cross for the characters concerned presents a monohybrid situation. The data are assembled in table 1 and will be discussed in the succeeding paragraphs.

A consideration of the data presented in table 1, taken as a whole, is only possible with respect to a few general conclusions because some of the cultures were from seeds sown in earth and others from seeds experimentally forced to a complete expression of their viability. It should be noted, however, that except for an occasional *oblonga*-like form or some dwarf rosettes, including *albida*, the plants were either *brevistylis* or *Lamarckiana*. The plants recorded as having died were usually weak seedlings which succumbed in the seed pans. There was a wide variation in the percentages of germination even when the wet seeds were subjected to pressure and then placed in Petri dishes. It might seem that the eleven sister plants of the F_1 varied in their power to produce viable seed and were therefore in this respect genetically different but it is possible that physiological conditions surrounding the individual plants led to these varying outputs of viable seeds. The data throw no light on this problem which can only be approached by physiological experiment. In three families (cultures 15.15 *c*, 16.15 *h* and 16.15 *i*) the ratios of *brevistylis* to *Lamarckiana* (1:15.5, 1:9.8 and 1:9) were very much less than the 1:3 ratios of our hypothesis, and in these cultures the germination percentages of 10.9, 19 and 15.8 were much below the higher percentages suggesting a correlation between low seed viability and smaller proportions of *brevistylis*. However, cultures SHULL 14169 and 16.15 *j* with rather low ratios of *brevistylis* to *Lamarckiana*, 1:6.5 and 1:5.4, but high percentages of germination, 31.2 and 33.3, did not support the hypothesis. Culture 15.15 *d*, from earth-sown seeds, with its very low percentage of germination (5.37) was too small a family to have much value in a discussion.

In a general consideration of the table I would emphasize the facts of a clear segregation of *brevistylis* from *Lamarckiana* in proportions for six of the eleven families close to the monohybrid ratio of 1:3. As the paper proceeds we shall find clearer evidence in support of the view that *brevistylis* is a simple recessive differing from *Lamarckiana* in a group of characters which are associated in inheritance.

Of particular interest are the data from cultures 15.41 and 15.42 since the F_1 parent plants bore some flowers with styles midway between the tips of the anthers and the top of the calyx tube (see p. 511) although in other respects resembling *Lamarckiana*. The genetical behavior of these two plants was quite the same as that of the sister plants

TABLE I

Segregation of *brevistylis* from *Lamarckiana* in the F_2 from the F_1 of *Lamarckiana S* $\times$ *brevistylis*.

Culture	F_1 parent plant	Seeds sown	Sown in	Percentage of germination	Number of <i>brevistylis</i>	Number of <i>Lamarckiana</i>	Other plants in the culture	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants
15.15 a	14.15 a	577 (4 capsules)	Petri dish	30.6*	—*	—	—	—
15.15 b	14.15 b	346 (4 capsules)	Earth	17	14	44		1:3.1 (1 plant died)
15.15 c	14.15 c	321 (4 capsules)	Earth	10.9	2	33		1:15.5 (2 plants died)
15.15 d	14.15 d	391 (4 capsules)	Earth	5.37	5	11		1:2.2 (5 plants died)
14169 SHULL	14.15 e	173	Earth	31.2	7	45	1 rosette	1:6.5 (1 plant died)
14177 SHULL	14.15 f	230	Earth	17.4	8	30	1 rosette	1:3.8 (1 plant died)
16.15 g	14.15 g	203 (2 capsules)	Petri dish	37.8	25	79	2 <i>oblonga</i> -like 1 narrow-leaved dwarf rosette	1:3.28 (4 plants died)
16.15 h	14.15 h	297 (2 capsules)	Petri dish	19	5	48	1 <i>oblonga</i> -like	1:9.8 (3 plants died)
16.15 i	14.15 i	208 (2 capsules)	Petri dish	15.8	3	26	1 <i>albida</i>	1:9 (3 plants died)
16.15 j	14.15 j	270 (2 capsules)	Petri dish	33.3	13	70	1 <i>albida</i>	1:5.4 (6 plants died)
15.41	14.15 y	163 (3 capsules)	Earth	34.3	16	36		1:2.2 (4 plants died)
15.42	14.15 z	97 (3 capsules)	Earth	27.8	7	19		1:2.7 (1 plant died)

* This culture was merely a test of the percentage of seed germination.

in the F_1 which showed a dominance of *Lamarckiana* characters, that is to say the segregation into *brevistylis* and *Lamarckiana* was just as sharp. In culture 15.41 only 5 of the 36 *Lamarckiana* plants were observed to produce occasional shorter-styled flowers and in culture 15.42 only 4 of the 19 *Lamarckianas* presented a similar behavior. Thus the tendency to develop shorter-styled flowers seemed no more marked in the *Lamarckiana* segregates of the F_2 than in the F_1 parent plants and I see no reason to believe that the peculiarity depends upon genetical factors different from those present in the usual F_1 hybrids of *Lamarckiana* $\times$ *brevistylis*. It is a peculiarity so variable in the degree of its expression and affecting so small a proportion of the flowers on a plant that its appearance seems more likely to be due to physiological causes. I found no material in the F_2 which would lead me to hope that the shorter-styled conditions might be fixed through selection.

A family in the F_3 (culture 16.3) from a selfed *Lamarckiana* plant in the F_2 of culture 15.15 *b* gave from 64 seedlings a total of 61 *Lamarckianas* and 2 narrow-leaved dwarfs indicating that this plant was pure *Lamarckiana*. Lack of garden space has so far prevented my testing a complete set of *Lamarckiana* plants from an F_2 generation. By our hypothesis $1/3$ of the plants in such a group should be pure *Lamarckiana* and $2/3$ should prove to have the constitution of the F_1 hybrid.

THE F_2 GENERATION FROM THE F_1 OF *BREVISTYLIS* $\times$ *LAMARCKIANA*

The records of the F_2 generations from *brevistylis* $\times$ *Lamarckiana* S are more satisfactory than the F_2 from the reciprocal F_1 cross described in the previous section. This is chiefly for the reason that all of the cultures were grown from wet seed subjected to pressure and germinated in Petri dishes, a treatment which resulted in the complete germination of all viable seeds. The four F_2 cultures were from F_1 plants in two different families and were related as sister plants as follows—16.1 *a*, I-3 and 16.1 *a*, II-3; 16.1 *b*, I-2, and 16.1 *b*, I-4. Therefore in a consideration of the genetical data, given in table 2, the F_2 cultures for purposes of comparison should be considered in pairs—17.51 and 17.52, 17.53 and 17.54.

As in the F_2 cultures previously described the segregation of *brevistylis* plants from *Lamarckiana* was perfectly sharp but the ratios were irregular. The most extreme range of variation, ratio 1:6.2 (culture 17.53) and ratio 1:2.5 (culture 17.54) is not as great as that shown in the cultures from reciprocal F_1 plants (see table 1) where the lowest ratio was 1:15.5 and the highest 1:2.2, both being from earth-sown cul-

TABLE 2

Segregation of *brevistylis* from *Lamarckiana* in the F_2 from the F_1 of *brevistylis* $\times$ *Lamarckiana* S.

Culture	F_1 parent plant	Seeds sown	Sown in	Percentage of germination	Number of <i>brevistylis</i>	Number of <i>Lamarckiana</i>	Other plants in the culture	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants
17.51	16.1 a, I-3	332 (2 capsules)	Petri dish	28.9	14	79		1:5.6 (3 plants died)
17.52	16.1 a, II-3	296 (2 capsules)	Petri dish	37.1	28	69	3 <i>oblonga</i> -like 1 <i>albida</i> 1 broad-leaved dwarf rosette	1:2.6 (8 plants died)
17.53	16.1 b, I-2	313 (3 capsules)	Petri dish	30.9	13	76	2 <i>oblonga</i> -like 1 <i>laevifolia</i> -like 1 <i>albida</i> 1 broad-leaved dwarf rosette	1:6.2 (3 plants died)
17.54	16.1 b, I-4	514 (3 capsules)	Petri dish	35.7	48	117	2 <i>oblonga</i> -like 1 <i>laevifolia</i> -like 2 <i>elliptica</i> 1 round-leaved dwarf rosette	1:2.5 (13 plants died)

TABLE 3

Segregation of *brevistylis* from *Lamarckiana* in double reciprocal crosses.

Culture	Seeds sown	Sown in	Percentage of germination	Number of <i>brevistylis</i>	Number of <i>Lamarckiana</i>	Other plants in the culture	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants
17.61 ($L \times b$) $\times$ ($b \times L$)	522 (3 capsules)	Petri dish	29.1	29	110	6 <i>oblonga</i> -like 2 <i>albida</i>	1:4 (5 plants died)
17.62 ($b \times L$) $\times$ ($L \times b$)	398 (3 capsules)	Petri dish	32.6	36	87	1 <i>oblonga</i> -like 1 <i>lata</i> 1 narrow-leaved dwarf rosette	1:2.5 (4 plants died)

tures. This closer approximation of the ratios is perhaps due to the fact that all of the seeds were fully germinated by the method of treatment with pressure.

It is interesting that the two families (cultures 17.51 and 17.53) with ratios of *brevistylis* to *Lamarckiana* much less than 1:3 (being 1:5.6 and 1:6.2) were families with lower percentages of germination (28.9 and 30.9) than in the cultures with which they were paired (cultures 17.52 and 17.54). This suggests very clearly a correlation between low seed viability and smaller proportions of *brevistylis*. It is noteworthy that in table 2 the higher ratios of *brevistylis* to *Lamarckiana* (cultures 17.52 and 17.54) are very close to 1:3 which was also true of the data assembled in table 1.

A larger number and greater variety of "mutants" were noted in these cultures than in those recorded in table 1, a fact perhaps due to complete germination. The plants listed as having died, generally refer to weak seedlings unable to live long.

THE DOUBLE RECIPROCAL CROSSES

Crosses were made both ways between a plant in the F_1 of *Lamarckiana* M $\times$ *brevistylis* (16.14 VI-3) and a plant in the F_1 of *brevistylis* $\times$ *Lamarckiana* S (16.1 b I-4) giving what DE VRIES has termed double reciprocal crosses. The same *brevistylis* stock was in both of the F_1 plants employed, but it happened that one of these F_1 hybrids was concerned with the race of *Lamarckiana* M and the other with the race *Lamarckiana* S. Therefore the double reciprocal crosses do not involve exactly equivalent lines of *Lamarckiana*, although, as has been stated, *Lamarckiana* M and S do not differ in their systematic characters and both lines run back to plants secured by DE VRIES at Hilversum.

The two cultures were 17.61, (*Lamarckiana* M $\times$ *brevistylis*) $\times$ (*brevistylis* $\times$ *Lamarckiana* S), and 17.62, (*brevistylis* $\times$ *Lamarckiana* S) $\times$ (*Lamarckiana* M $\times$ *brevistylis*). On an assumption that the same types of fertile gametes are formed through both pollen and ovules of the parent F_1 hybrid plants the generations from a double reciprocal cross should give the same segregation as F_2 generations from either F_1 parent of the cross. Thus the F_1 *Lamarckiana* $\times$ *brevistylis* and the F_1 *brevistylis* $\times$ *Lamarckiana* by our hypothesis develop gametes *b* and *L*, and when each is selfed the combinations in the F_2 generations under conditions of uniform fertility would be 1 *bb*:2 *bL*:1 *LL* or the phenotypic ratio of 1 *brevistylis*:3 *Lamarckiana*. These same F_1 hybrids when crossed to give double reciprocals would therefore be expected to

present the same segregation, namely 1 *brevistylis* : 3 *Lamarckiana*, since each develops the same types of gametes. The breeding data of the double reciprocal crosses are given in table 3 (p. 516).

The expectations of our hypothesis were supported by the breeding results since in both double reciprocals the proportion of *brevistylis* to *Lamarckiana* was close to the ratio 1 *brevistylis* : 3 *Lamarckiana*. The departure of the ratios in these two cultures of double reciprocals from the 1 : 3 of our hypothesis is perhaps not greater than might be expected in small cultures, but it is interesting to find again a possible correlation of a lower germination percentage (29.1 in culture 17.61) with a smaller proportion of *brevistylis* (1 : 4). As in the cultures previously described, the plants listed as having died usually refer to weak seedlings.

BACK CROSSES OF THE F_1 RECIPROCAL HYBRIDS (*LAMARCKIANA* $\times$ *BREVISTYLIS*)
AND (*BREVISTYLIS* $\times$ *LAMARCKIANA*) TO THE PARENT STOCK

Back crosses of the two F_1 reciprocals (*Lamarckiana* $\times$ *brevistylis*) and (*brevistylis* $\times$ *Lamarckiana*) were made in all possible combinations with the parent stock giving the eight cultures listed below, to which DE VRIES's convenient descriptive terms of sesquireciprocal and iterative are also applied.

17.63 Iterative	(<i>Lamarckiana</i> M $\times$ <i>brevistylis</i>) $\times$ <i>brevistylis</i>
17.64 Sesquireciprocal	<i>brevistylis</i> $\times$ (<i>Lamarckiana</i> M $\times$ <i>brevistylis</i>)
17.65 Sesquireciprocal	(<i>Lamarckiana</i> M $\times$ <i>brevistylis</i>) $\times$ <i>Lamarckiana</i> M
17.66 Iterative	<i>Lamarckiana</i> M $\times$ (<i>Lamarckiana</i> M $\times$ <i>brevistylis</i>)
17.67 Sesquireciprocal	(<i>brevistylis</i> $\times$ <i>Lamarckiana</i> S) $\times$ <i>brevistylis</i>
17.68 Iterative	<i>brevistylis</i> $\times$ (<i>brevistylis</i> $\times$ <i>Lamarckiana</i> S)
17.69 Iterative	(<i>brevistylis</i> $\times$ <i>Lamarckiana</i> S) $\times$ <i>Lamarckiana</i> S
17.70 Sesquireciprocal	<i>Lamarckiana</i> S $\times$ (<i>brevistylis</i> $\times$ <i>Lamarckiana</i> S)

It should be noted that the two races of *Lamarckiana*, M and S, were concerned in the series, but that each back cross involved the same race. Breeding data for the eight crosses are given in table 4.

The results of the back crosses are exactly as would be expected from our hypothesis as far as the character of the progeny is concerned. Back crosses to *Lamarckiana* whether the pollen parent (cultures 17.65 and 17.69) or the ovule parent (cultures 17.66 and 17.70) gave progenies with no plants of *brevistylis*, thus showing the dominance of *Lamarckiana* so conspicuous in the F_1 generations. Back crosses to *brevistylis*, the recessive, gave perfectly clear segregation of *brevistylis* (cultures 17.63, 17.64, 17.67 and 17.68). Fertilization of *brevistylis* by the

TABLE 4
Back crosses of the F₁ reciprocal hybrids (Lamarckiana × brevistylis) and (brevistylis × Lamarckiana) to the parent stock.

Culture	Seeds sown	Sown in	Percentage of germination	Number of <i>brevistylis</i>	Number of <i>Lamarckiana</i>	Other plants in the culture	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants
17.53 ($L \times b$) × b Iterative	427 (2 capsules)	Petri dish	25.5	42	58	3 <i>oblonga</i> -like 1 <i>albida</i> 1 broad-leaved dwarf rosette	1:1.5 (4 plants died)
17.64 $b \times (L \times b)$ Sesquiterciprocal	76 (15 capsules)	Petri dish	32.8	4	4		1:1 (17 plants died)
17.65 ($L \times b$) × L Sesquiterciprocal	314 (2 capsules)	Petri dish	43.9	none	127	1 <i>oblonga</i> -like 2 <i>nanella</i> 1 <i>albida</i>	0:1 (7 plants died)
17.66 $L \times (L \times b)$ Iterative	244 (2 capsules)	Petri dish	25.8	none	58	1 <i>nanella</i>	0:1 (4 plants died)
17.67 ($b \times L$) × b Sesquiterciprocal	295 (3 capsules)	Petri dish	35.5	36	61	1 <i>oblonga</i> -like 1 <i>rubrinervis</i> 1 narrow-leaved dwarf rosette	1:1.7 (5 plants died)
17.68 $b \times (b \times L)$ Iterative	37 (18 capsules)	Petri dish	37.8	1	8		1:8 (5 plants died)
17.69 ($b \times L$) × L Iterative	359 (3 capsules)	Petri dish	51.8	none	180	1 <i>laevifolia</i> -like 1 <i>lata</i>	0:1 (4 plants died)
17.70 $L \times (b \times L)$ Sesquiterciprocal	513 (3 capsules)	Petri dish	33.5	none	105	3 <i>Lamarckiana</i> -like rosettes 1 <i>albida</i>	0:1 (3 plants died)

F₁ hybrids proved difficult of accomplishment because of its low seed fertility and the yield of seeds was very small. Thus culture 17.64 came from 76 seeds, the contents of 15 capsules, and culture 17.68 came from 37 seeds, the contents of 18 capsules. In both of these cultures the mortality among seedlings and young plants was very great (68 percent for culture 17.64 and 35.8 percent for culture 17.68) and because of this fact together with the small numbers in the cultures the ratios of *brevistylis* to *Lamarckiana*, 1:1 and 1:8, have little value for our discussion.

For the other two back crosses with *brevistylis* as the pollen parent, cultures 17.63 and 17.67, we have fairly large families and ratios of *brevistylis*, 1:1.5 and 1:1.7, which approximate the ratio of 1:1 expected in a cross of a monohybrid back to a recessive parent stock, when there are no complications of differential mortality among gametes or zygotes. It seems significant that the numbers of *brevistylis* fall below expectations and a failure of *brevistylis* to reach expected proportions or to go very slightly beyond these proportions is generally characteristic of the results recorded in tables 1, 2, and 3 as well as in table 4. For the cultures here described it seems possible that a mortality, higher for *brevistylis*, may be responsible for the departures from expected genetical ratios. This problem will receive further consideration in the discussion.

OBSERVATIONS ON A SELFED LINE OF *OENOTHERA BREVISTYLIS*

As previously stated my material of *brevistylis* came to me from Professor DE VRIES through seeds of an F₁ *Lamarckiana* × *brevistylis*. From these seeds a culture was grown in 1913 which showed the usual F₂ segregation into *brevistylis* and *Lamarckiana* (culture 13.15). Pollination of *Lamarckiana* S from one of the *brevistylis* plants gave me in 1914 an F₁ generation (culture 14.15) and selfing eleven of these F₁ plants (14.15 b, c, d, e, f, g, h, i, j, y and z) made possible in 1915 and 1916 the F₂ cultures described in table 1.

Among the *brevistylis* plants in these F₂ families some were noted as having a much larger proportion of flowers with longer styles and more perfectly formed stigmas than seemed usual for *brevistylis* (fig. 7). Thus an opportunity was presented to test the possibility of improving the style length of *brevistylis* and its fertility by selfing flowers on selected plants showing a greater variation in the direction of normal *Oenothera* styles and stigmas. Four plants of *brevistylis* were chosen (15.15 b, I-1; 15.15 b, I-4; 15.15 b, II-5; 15.15 d, I-1), favorable flowers were self-pollinated and from their seed were grown the cultures of selfed *brevistylis* listed in table 5.

TABLE 5

Culture	Parent <i>brevistylis</i> plant	Seeds sown	Sown in	Percentage of seeds with contents	Number of seedlings	Plants reaching maturity, all <i>brevistylis</i>
16.2 a	15.15 b, I-1	55 15 capsules	Petri dish	45	21	15
16.2 b	15.15 b, I-4	130 21 capsules	Petri dish	46	45	43
16.2 c	15.15 b, II-5	78 14 capsules	Petri dish	35.9	18	18
16.2 d	15.15 d, I-1	33 5 capsules	Petri dish	48.5	16	16

FIGURE 7.—At the right, a flower of *brevistylis* with stigma at a much higher level than is usual for this species (plant 15.15 b, I-1). A selfed line of plants showing large proportions of such flowers is being carried along to test the possibility of improving through selection the style length and fertility of *brevistylis*.

TABLE 6

Performance in the first generation of 8 plants of brevistylis from selfed parents selected for their tendency to produce long-styled flowers.

Plants	Observations	Flowers observed	Long styles	Short styles	Percentage of long styles	Yield of seed from selfed flowers
16.2 b, II-3	July 18-Aug. 3	26	21	5	80	18 flowers selfed gave 10 shriveled capsules with 12 seeds
16.2 b, III-3	July 18-Aug. 2	16	9	7	56	12 flowers selfed gave 7 capsules with 18 seeds
16.2 b, VI-5	July 18-Aug. 4	24	15	9	62	16 flowers selfed gave 9 capsules with 19 seeds
16.2 b, VII-1	July 18-Aug. 2	16	13	3	81	18 flowers selfed gave 12 capsules with 36 seeds
16.2 d, I-2	July 18-Aug. 1	25	20	5	80	21 flowers selfed gave 11 capsules with 52 seeds
16.2 d, II-4	July 18-Aug. 2	15	13	2	86	17 flowers selfed gave 9 capsules with 31 seeds
16.2 d, IV-1	July 18-Aug. 5	22	21	1	95	21 flowers selfed gave 13 capsules with 55 seeds
16.2 d, IV-4	July 18-Aug. 3	19	19	0	100	14 flowers selfed gave 8 capsules with 104 seeds

TABLE 8

Performance in the second generation of *brevistylis* in a selfed line from parents selected for their tendency to produce long-styled flowers.

Plants	Observations	Flowers observed	Long styles	Medium styles	Short styles	Percentage of long styles	Yield of seed from selfed flowers
17.2, I-1	June 28-July 28	37	11	11	15	29.7	
* I-2	" 30- " 28	26	18	5	3	69.2	{ 13 flowers selfed gave 2 capsules with 7 seeds
* I-3	" 28- " 28	31	17	11	3	54.8	{ 10 flowers selfed gave 2 capsules with 5 seeds
I-4	" 28- " 28	37	14	8	15	37.8	
I-5	July 3- " 28	28	12	11	5	42.8	
* II-1	June 28- " 28	34	22	11	1	64.7	{ 17 flowers selfed gave no capsules
II-2	" 28- " 28	37	16	12	9	43.2	
II-3	" 29- " 28	34	16	15	3	47	
II-4	" 30- " 28	27	6	9	12	22.2	
II-5	July 3- " 28	27	8	12	7	29.6	
* III-1	June 28- " 28	34	25	6	3	73.5	{ 19 flowers selfed gave 2 capsules with 4 seeds
III-2	" 29- " 28	29	9	12	8	31	
* III-3	" 30- " 28	29	19	5	5	65.5	{ 15 flowers selfed gave 6 capsules with 21 seeds
III-4	" 28- " 28	32	11	12	9	34.3	
III-5	July 2- " 28	27	2	10	15	7.4	
IV-1	June 28- " 28	33	13	7	13	39.3	
IV-2	" 29- " 28	32	3	17	12	9.3	
IV-3	" 29- " 28	35	7	14	14	20	
IV-4	" 29- " 28	26	4	6	16	15.3	
IV-5	July 1- " 28	29	11	11	7	37.9	
* V-1	June 30- " 28	31	19	9	3	61.3	{ 24 flowers selfed gave 11 capsules with 54 seeds. Largest capsules 9-10 mm long
V-2	" 29- " 28	30	5	10	15	16.6	
V-3	" 30- " 28	34	10	13	11	29.4	
V-4	" 30- " 28	28	8	15	5	28.5	
V-5	July 3- " 28	28	3	10	15	10.7	
VI-1	" 1- " 28	27	6	10	11	22.2	
VI-2	June 29- " 28	32	4	14	14	12.5	
* VI-3	July 1- " 28	26	20	5	1	76.9	{ 11 flowers selfed gave no capsules
VI-4	" 3- " 28	26	9	8	9	34.6	
* VII-1	" 2- " 28	28	21	6	1	75	{ 18 flowers selfed gave no capsules
VII-2	" 2- " 28	25	9	12	4	36	
* VII-3	" 1- " 28	33	17	13	3	51.5	{ 16 flowers selfed gave no capsules
VII-4	" 1- " 28	31	0	12	19	0	

These cultures of selfed *brevistylis* (16.2 *a*, *b*, *c* and *d*) presented a wide range of variation in their tendency to produce flowers with styles longer than is usual to *brevistylis*. Consequently the performance of individual plants was tested before making selections to self for the next generation. The test consisted in recording the length of style in a series of flowers opening along the main stem of a plant over a definite period of time. In these cultures the styles were recorded as *long* when they extended well above the top of the calyx tube and as *short* if the stigma was at or below the level of the calyx tube. The record of performance as observed for eight plants is given in table 6.

From its record of performance, as shown in table 6, plant 16.2 *d*, IV-4 was picked as the parent for a second generation of this selfed line of *brevistylis*. It set the usual small quantity of seeds when selfed, 104 seeds from 8 capsules, and from these there was grown in 1917 a culture with a record as follows:

TABLE 7

Culture	Parent <i>brevistylis</i> plant	Seeds sown	Sown in	Percentage of ger- mination	Number of seed- lings	Plants reaching maturity, all <i>brevistylis</i>
17.2	16.2 <i>d</i> , IV-4	104 8 capsules	Petri dish	34.6	36	33

The performance of the 33 plants in this second generation of selfed *brevistylis* with respect to their production of longer-styled flowers was recorded as in the case of the first generation of selfed *brevistylis* by observing for each plant a series of flowers as they opened over a certain length of the main stem. The classification was, however, modified so that three grades were noted instead of two, *long* when the styles reached the lower ends of the anthers or extended above, *medium* when the stigma lay between the lower ends of the anthers and top of the calyx tube, *short* when the stigma was at or below the top of the calyx tube. The record of the performance of this second generation of selfed *brevistylis* is given in table 8.

An examination of table 8 will show at once a very marked regression of the second generation of selfed *brevistylis* from the performance of its parent plant 16.2 *d*, IV-4 (table 6), which while under observation presented no short-styled flowers of our classification and a yield of 104 seeds from 8 capsules, an average of 13 seeds to a capsule. The plants making the best records in the second generation were *17.2, I-2; *I-3;

*II-1; *III-1; *III-3; *V-1; *VI-3; *VII-1; *VII-3. These plants were selfed by carefully selecting flowers with longer styles and better-developed stigmas but the results were disappointing as to the yield of seeds (see table 8). Some of the plants developed no seeds, the ovaries withering; others developed occasional small capsules with few seeds. The best yield was from plant *17.2, V-1, from which, following 24 selfings, 11 capsules were obtained with a total of 54 seeds, an average of about 5 seeds to a capsule. No plant in this second generation equaled the record of the parent in style length and seed production and most of the plants fell far behind its performance.

I expect to carry this selfed line through further generations selecting for length of style and for seed production, since fertility is of course necessary to progress of the study. The material presents a character (length of style and development of stigma lobes) highly variable and would seem to be excellent for a test of the power of selection to achieve results in a close-pollinated line.

DISCUSSION

Before taking up as a whole the data on the ratios of *brevistylis* plants segregated from the crosses with *Lamarckiana* the following facts should be remembered in their broad relations to the problem. (1) There is presented in the F_1 an almost perfect dominance of *Lamarckiana* characters when this plant is crossed with *brevistylis* or with an F_1 hybrid of *Lamarckiana* and *brevistylis*. (2) There is a perfectly clean segregation of *brevistylis* plants in the F_2 from the reciprocal F_1 generations of crosses between *brevistylis* and *Lamarckiana*, from the double reciprocal crosses $(L \times b) \times (b \times L)$ and $(b \times L) \times (L \times b)$, and from the back crosses of the F_1 hybrids to *brevistylis*. (3) *Brevistylis* shows itself to be a recessive, by breeding true, by the dominance of *Lamarckiana* in crosses, and by segregating from appropriate crosses in ratios approximating those to be expected of monohybrids.

Mention should first be made of other variants from *Lamarckiana* which appeared in the cultures besides *brevistylis*. They are listed in tables 1, 2, 3 and 4, and it is interesting to note that the earth-sown cultures quite failed to bring them out; they only appeared in cultures from seed forced to complete germination. Most of the variants were easily identified with "mutants" described by DE VRIES from *Lamarckiana*. Thus *albida* (9), *nanella* (3), *lata* (2), and *rubrinervis* (1) were readily recognized. A type close to *oblonga*, but generally larger, was most common (22) and a narrow-leaved form similar to *laevifolia* (3)

was also present. Narrow-leaved dwarfs (5) resembling *elliptica* appeared and there were also broad-leaved dwarf rosettes (4). Since none of these variants show any of the *brevistylis* peculiarities they have been grouped with *Lamarckiana* in calculating the proportions of *brevistylis* in the cultures.

The most important problem presented is an understanding of why the ratios of *brevistylis* to *Lamarckiana* and other plants in the cultures should present so great a range of variation in the results offered for comparison. Two possible explanations suggest themselves. (1) It is possible that sister plants of the same cross may differ in their genetical constitutions and for this reason some may throw *brevistylis* in larger or smaller proportions than others. (2) Or, it is possible that variations in the ratios of *brevistylis* may result from environmental factors, as for example malnutrition, which operate to prevent the full expression of zygotes that normally should give viable seeds of *brevistylis*.

I am working on the hypothesis that the latter explanation is more likely to account for irregularities of ratios and shall discuss the data here presented from that standpoint. It should, however, first be noted that there are sure to be uncertainties of data with material in which some plants in the cultures die generally as seedlings, frequently because they are unable to throw off their seed coats. This condition can only be fully appreciated through cultures in which the seeds are experimentally germinated and the fate and condition of all are fully recorded. Such data are obviously impossible to obtain in earth-sown cultures. This mortality must increase or diminish the ratios, as recorded, according to the nature of the dead plants whether or not *brevistylis*. Therefore, variations from expected ratios are more likely to occur than in material in which generations can be brought to maturity with little or no loss by death.

There are assembled in table 9 the cultures which presented *brevistylis* as a segregate in ratios close to expectations on the hypothesis of a monohybrid situation; and in table 10 are brought together the cultures in which the ratios of *brevistylis* were far from expectations. The full data for all of the cultures are given in tables 1-4 to which reference is made in the first column, and this data must be considered in an examination of tables 9 and 10. Thus in table 9 cultures 15.15 *d* and 17.64, and in table 10 culture 17.68, give ratios of little significance (apart from the important fact of a segregation) because the cultures were small and the mortality high. There are no indications from these records in particular that ratios from cultures in Petri dishes are less irregular

TABLE 9

Cultures presenting the segregation of brevistylis in proportions close to expectations on the hypothesis of a monohybrid situation.

Culture	Its character	Seeds sown in	Percentage of germination	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants	Ratio to be expected by the hypothesis
15.15 <i>b</i> (Table 1)	F_2 from $L \times b$	Earth	17	1:3.1	1:3
15.15 <i>d</i> (Table 1)	F_2 from $L \times b$	Earth	5.37	1:2.2	1:3 (Small culture and high mortality make data of little value)
SHULL 14177 (Table 1)	F_2 from $L \times b$	Earth	17.4	1:3.8	1:3
16.15 <i>g</i> (Table 1)	F_2 from $L \times b$	Petri dish	37.8	1:3.28	1:3 (High percentage of germination)
15.41 (Table 1)	F_2 from $L \times b$	Earth	34.3	1:2.2	1:3
15.42 (Table 1)	F_2 from $L \times b$	Earth	27.8	1:2.7	1:3
17.52 (Table 2)	F_2 from $b \times L$	Petri dish	37.1	1:2.6	1:3 (High percentage of germination)
17.54 (Table 2)	F_2 from $b \times L$	Petri dish	35.7	1:2.5	1:3 (High percentage of germination)
17.61 (Table 3)	Double reciprocal $(L \times b) \times (b \times L)$	Petri dish	29.1	1:4	1:3 (Fair percentage of germination)
17.62 (Table 3)	Double reciprocal $(b \times L) \times (L \times b)$	Petri dish	32.6	1:2.5	1:3 (Fair percentage of germination)
17.63 (Table 4)	Iterative $(L \times b) \times b$	Petri dish	25.5	1:1.5	1:1 (Fair percentage of germination)
17.64 (Table 4)	Sesquiereciprocal $b \times (L \times b)$	Petri dish	32.8	1:1	1:1 (Small culture and very high mortality make data of little value)
17.67 (Table 4)	Sesquiereciprocal $(b \times L) \times b$	Petri dish	35.5	1:1.7	1:1 (High percentage of germination)

than those from earth-sown cultures but of course the percentages of germination for the former have significance as accurate tests of seed sterility while the percentages for the latter may or may not be approximations to the facts of seed viability.

An examination of table 9 (with ratios of *brevistylis* close to expectations) will show that the percentages of germination in Petri dish cultures is generally high. Of the eight cultures in Petri dishes only two showed a germination below 32 percent, these being cultures 17.61 (29.1) and 17.63 (25.5) both of which present *brevistylis* in proportions lower than expectations. The other six cultures range from 32.6 percent (culture 17.62) to 37.8 percent (culture 16.15 g).

In table 10 with ratios of *brevistylis* far from expectations, the percentages of germination recorded for Petri dish cultures is much lower than for the cultures assembled in table 9. Excluding culture 17.68 where the ratio, as already noted, has little significance, there are five cultures to be considered. Among these the highest percentage of germination is 33.3 (culture 16.15 j) and the other four cultures are below 31 percent with the two lowest percentages 15.8 (culture 16.15 i) and 19 (culture 16.15 h). Of the greatest significance perhaps is the fact that *brevistylis* was in its lowest proportions to *Lamarckiana*, 1:9 and 1:9.8, in cultures 16.15 i and 16.15 h where the percentages of germination were the lowest (15.8 and 19) recorded for Petri dish cultures.

The data of tables 9 and 10 may be examined from another angle. There are only three Petri dish cultures (table 9, cultures 17.52, 17.54 and 17.62) in which *brevistylis* appeared in proportions higher than expectations and in these the ratios vary from expectations by no more than 0.5. This perhaps represents a departure no greater than might be expected in material so difficult to bring to complete expression as these *Oenotheras*. On the other hand all of the other nine cultures in Petri dishes (17.64 and 17.68, excluded) present *brevistylis* in lower proportions than expectations and the departures range from 0.28 (16.15 g) to 6.8 (16.15 h) with eight cultures showing a variance above 0.5. In five of these nine cultures (table 10) the variance from expected ratios ranged from 2.4 to 6.8. On the hypothesis of a monohybrid situation in these crosses the departures from expected ratios is only marked where *brevistylis* appears in fewer numbers and this condition appears to be correlated with lower percentages of germination, such as might indicate a differential mortality working against the formation of *brevistylis* zygotes or against their development.

TABLE 10

Cultures presenting the segregation of brevistylis in proportions far from expectations on the hypothesis of a monohybrid situation.

Culture	Its character	Seeds sown in	Percentage of germination	Ratio of <i>brevistylis</i> to <i>Lamarckiana</i> and other plants	Ratio to be expected by the hypothesis
15.15 c (Table 1)	F ₂ from $L \times b$	Earth	10.9	1:5.5	1:3
SHULL 14169 (Table 1)	F ₂ from $L \times b$	Earth	31.2	1:6.5	1:3
16.15 h (Table 1)	F ₂ from $L \times b$	Petri dish	19	1:9.8	1:3 (Low percentage of germination)
16.15 i (Table 1)	F ₂ from $L \times b$	Petri dish	15.8	1:9	1:3 (Low percentage of germination)
16.15 j (Table 1)	F ₂ from $L \times b$	Petri dish	33.3	1:5.4	1:3 (Fair percentage of germination)
17.51 (Table 2)	F ₂ from $b \times L$	Petri dish	28.9	1:5.6	1:3 (Fair percentage of germination)
17.53 (Table 2)	F ₂ from $b \times L$	Petri dish	30.0	1:6.2	1:3 (Fair percentage of germination)
17.68 (Table 4)	Iterative $b \times (b \times L)$	Petri dish	37.8	1:8	1:1 (Small culture and high mortality make data of little value)

TABLE II

Petri dish cultures arranged in order beginning with those in which the proportions of brevistylis are far below expectations. Note the marked rise in the percentages of germination among the cultures in which the ratios of brevistylis approach expectations.

Cultures	For full data see	Recorded ratios of <i>brevistylis</i>	Ratio of expectations	Percentages of germination	Remarks
17.68	Table 10	1:8	1:1	37.8	{ Small numbers and high mortality make the data for culture 17.68 of little value (see table 4).
16.15 <i>h</i>	Table 10	1:9.8	1:3	19	
16.15 <i>i</i>	Table 10	1:9	1:3	15.8	
17.53	Table 10	1:6.2	1:3	30.9	
17.51	Table 10	1:5.6	1:3	28.9	
16.15 <i>j</i>	Table 10	1:5.4	1:3	33.3	
17.61	Table 9	1:4	1:3	29.1	
17.67	Table 9	1:1.7	1:1	35.5	{ Small numbers and very high mortality make the data for culture 17.64 of little value (see table 4).
17.63	Table 9	1:1.5	1:1	25.5	
16.15 <i>g</i>	Table 9	1:3.28	1:3	37.8	
17.64	Table 9	1:1	1:1	32.8	
17.52	Table 9	1:2.6	1:3	37.1	
17.54	Table 9	1:2.5	1:3	35.7	
17.62	Table 9	1:2.5	1:3	32.6	

Arranging the Petri dish cultures in order beginning with those in which the proportions of *brevistylis* are lowest we find that the percentages of germination at the beginning of the series are in general much lower than those recorded for the cultures in which *brevistylis* is close to expectations or in somewhat greater proportions. The list is given in table II and the marked rise in germination percentages among the cultures in which the ratios of *brevistylis* approach expectations is evident.

A list of earth-sown cultures similarly arranged is presented in table 12. In this table there will not be found so regular a correlation of low proportions of *brevistylis* with low percentages of germination as in the list of Petri dish cultures (table II), but this does not seem to me surprising in view of my observations on the wide variation in the rate of germination exhibited by earth-sown cultures of *Oenotheras* (DAVIS 1915 *b*, p. 237; 1917, pp. 168, 177). In earth-sown cultures incomplete germination may readily result in marked departures from correct ratios according as various physiological factors, about which we know very little, may favor somewhat either *brevistylis* or *Lamarckiana*, or work against the germination of their viable seeds.

TABLE 12

Earth-sown cultures arranged in order, beginning with those in which the proportions of brevistylis are far below expectations. Note that there is not present so striking a correlation of low proportions of brevistylis with low percentages of germination as shown in table 11, a fact possibly due to irregularities and variations in the rates of germination under the physiological conditions of earth-sown cultures.

Cultures	For full data see	Recorded ratios of brevistylis	Ratios of expectations	Percentages of germination	Remarks
15.15 c	Table 10	1:15.5	1:3	10.9	
SHULL					
14169	Table 10	1:6.5	1:3	31.2	
SHULL					
14177	Table 9	1:3.8	1:3	17.4	Small numbers and high mortality make the data for culture 15.14 d of little value (see table 1).
15.15 b	Table 9	1:3.1	1:3	17	
15.42	Table 9	1:2.7	1:3	27.8	
15.41	Table 9	1:2.2	1:3	34.3	
15.15 d	Table 9	1:2.2	1:3	5.37	

This finishes the consideration of the data presented in this paper. It might be more extensive and it is unavoidably mixed in its nature since both earth-sown cultures and cultures from complete germination in Petri dishes are considered, but I trust that its presentation is justified together with the working hypothesis which is offered for the interpretation of the results. This hypothesis presupposes that the group of associated characters which distinguishes *brevistylis* from *Lamarckiana* is recessive to the characters of *Lamarckiana* and that the situation in F_1 generations from crosses of *brevistylis* with *Lamarckiana* is that of a monohybrid. The hypothesis also assumes that the segregation of *brevistylis* which appears in the F_2 , would follow when fully expressed the simple Mendelian ratio of 1:3, and that the back cross of the F_1 to the recessive *brevistylis* would give the familiar ratio of 1:1 if undisturbed by extraneous factors. Proportions of *brevistylis* lower than expectations on this hypothesis are believed to be due to a differential mortality affecting the *brevistylis* segregates and expressed by lower percentages of germination in those cultures that exhibit such departures from the expected ratios.

There is an experimental method of attack on the problems developed by the data of this paper for which plans have been laid and preliminary steps taken. Plants of the F_1 generation of reciprocal crosses between *brevistylis* and *Lamarckiana* have so long a flowering season that it is

possible to self and collect a harvest of ripened seeds by the middle of August. The plants at this time in the summers of Philadelphia are still in fine flower and a second harvest of selfed seed may easily be obtained before the end of the season. Thus it is possible to experiment on selected plants, the breeding behavior of which may be established by cultures of their seeds ripened earlier in a flowering season. Certain strong plants from reciprocal F_1 crosses were chosen in the summer of 1917, selfed, and their seed ripened in the early part of the season when the parent plants were in full vegetative vigor. After harvesting the seed these same plants were subjected by the removal of branches and leaves to a treatment certain to place the selfed seeds of a second harvest under conditions of malnutrition in varying degrees of severity. Will the proportions of *brevistylis* in F_2 cultures from seeds of the second harvest (subjected to malnutrition) be found to differ markedly from the proportions in cultures of seeds ripened on the same plants early in the season under conditions of very healthy vegetative vigor?

SUMMARY

1. The F_1 generations of reciprocal crosses between *Oenothera brevistylis* and *Oe. Lamarckiana* are uniform and essentially indistinguishable from one another. The characters of *Lamarckiana* are strongly dominant and the hybrids may be separated from *Lamarckiana* only when measurements of strongly contrasted characters of the parents are compared. Then the influence of the *brevistylis* parent may appear in styles not quite so long as those of *Lamarckiana*, in leaves and bracts somewhat broader, bud cones a little thicker, and sepal tips somewhat shorter.

2. The F_2 generations from reciprocal F_1 hybrids present a perfectly sharp segregation of *brevistylis* plants from *Lamarckiana* in proportions approximating the monohybrid Mendelian ratio of 1 *brevistylis* : 3 *Lamarckiana*. Thus the distinguishing characters of *brevistylis* are inherited in closely associated relations, behaving as a unit. Marked departures from this 1 : 3 ratio are in the direction of lower proportions of *brevistylis* and appear to be correlated with smaller percentages of viable seeds, indicating that a differential mortality may work against the appearance of *brevistylis* and thus modify the Mendelian ratio of expectation (tables 1, 2, 9, 10).

3. Double reciprocal crosses, as would be expected, gave ratios of *brevistylis* to *Lamarckiana* close to 1 : 3 and here also a smaller proportion of *brevistylis* was accompanied by a lower percentage of seed germination (tables 3, 9).

4. Back crosses of the reciprocal F_1 hybrids to the parent *Lamarckiana* (table 4) gave progenies with the characters of *Lamarckiana* as would be expected from the dominance of *Lamarckiana* shown in the F_1 generations of crosses between the parent species.

5. Back crosses of the reciprocal F_1 hybrids to the parent *brevistylis* present the same clear segregation of *brevistylis* exhibited by the F_2 generations and by the double reciprocal crosses. The proportions of *brevistylis* to *Lamarckiana* approximated the ratio of 1 : 1 to be expected when a monohybrid is crossed with its recessive parent stock (tables 4, 9).

6. Variants appeared in the cultures from seeds forced to complete germination but were not noted in earth-sown cultures. Some of these were clearly the "mutants," *albida*, *nanella*, *lata*, *rubrinervis*, and narrow-leaved dwarfs close to *elliptica*. Others were similar to *oblonga* and *laevifolia*. Certain broad-leaved dwarf rosettes were also present.

7. *Oenothera brevistylis* breeds true in agreement with its evidently recessive nature.

8. A selfed line of *brevistylis* has been started, the plants of which show a wide range in the length of style, perfection in the development of stigma lobes, and yield of seed. Selection towards a more perfect expression of these organs and a higher yield of seed has been practiced for two generations. The results so far have not led to improvement.

9. The breeding data indicate that crosses between *brevistylis* and *Lamarckiana* result in a simple monohybrid situation. On this assumption the hypothesis is proposed that the variations in the expected ratios of *brevistylis* as a segregate in the F_2 generations, double reciprocal crosses, and back crosses of the F_1 to *brevistylis* result from environmental factors, as perhaps malnutrition, which operate to prevent the expression of zygotes that normally should give viable seeds of *brevistylis*. Such factors if operative should on the hypothesis lead to lower percentages of viable seeds, and apparently lower percentages of viable seeds are correlated with lower proportions of *brevistylis* as a segregate. Steps have been taken to test this hypothesis by an experimental method of attack.

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