MALNUTRITION AS A CAUSE OF IRREGULARITIES IN THE SEGREGATION OF *OENOTHERA BREVISTYLIS* FROM CROSSES WITH *OENOTHERA LAMARCKIANA*¹

BRADLEY MOORE DAVIS

University of Michigan, Ann Arbor, Michigan

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In a recent paper the writer (DAVIS 1918) assembled a considerable body of data in support of the conclusions of DE VRIES that the characters distinguishing *Oenothera brevistylis* from *Oe. Lamarckiana* are closely linked and segregate from crosses with *Lamarckiana* on the basis of a simple monohybrid situation. Thus the characters of *brevistylis* are recessive to those of *Lamarckiana* in reciprocal F_1 generations; they segregate sharply in F_2 generations from selfed F_1 plants, and also in double reciprocals, in proportions close to the monohybrid ratio of 1 : 3; they segregate in back-crosses of the F_1 plants to the recessive *brevistylis* in proportions close to the monohybrid ratio of 1 : 1.

There were, however, in the results, some apparently rather constant variations from expectations in the ratios, and smaller proportions of *brevistylis* plants seemed to be correlated with a lowered seed viability, indicating that conditions which reduced the percentages of viable seeds also lowered the proportions of *brevistylis* plants. It was suggested that environmental factors such as malnutrition might eliminate some of the zygotes and embryos which under more favorable conditions, would live to develop into *brevistylis* plants. An experimental method of attack on the problem was formulated and has been tried out with the results presented in this paper.

The plan of experimentation was as follows: When cultures of an F_1 generation of reciprocal crosses between *brevistylis* and *Lamarckiana* are started in the hot-house and set out early in the spring, plants may be selfed and a harvest of seed collected by the middle of August. The plants at this time are still in fine flower and in the long summers of Philadelphia a second harvest of selfed seed may easily be obtained before the plants

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are killed by frost. Thus it is possible to experiment on selected plants the breeding behavior of which may be established by cultures from seed ripened earlier in the flowering season. Following this procedure plants from reciprocal F_1 crosses were selfed early in July and their seed collected about the middle of August. After this first harvest the same plants were again selfed and, following fertilization, the leaves and branches were removed from the plants so that the second harvest of seed was ripened under conditions of malnutrition.

A first set of experiments started in 1917 showed that it is not easy to hinder the production of seed in these plants, for the removal of as many as three-quarters of the leaves and branches did not appreciably affect the ratio of *brevistylis* segregates in the F_2 from seeds ripened under what might seem to be adverse conditions. It is only when all leaves and all branches are removed and the main shoot with its few selfed ovaries stands as a bare stem that marked modifications of the proportions of *brevistylis* and percentages of germination became apparent. This paper describes results from a series of experiments with F_1 plants made in 1919 in Philadelphia, the F_2 generation being grown at Ann Arbor, Michigan, in 1920. In this series all of the leaves and branches were removed from the plants under experiment.

Table 1 gives the plants of the reciprocal F_1 generations upon which experiments were made, the number of flowers in each second selfing, and the number and condition of the capsules resulting from these selfings at the death of the plants in September. It is to be noted that the plants rarely matured all of the capsules from the second selfings, many of them shriveling up, and also that the capsules actually ripened were frequently shrunken or small in size.

The F_2 generations from selfed seed of the F_1 plants listed in table 1 were then two in number for each plant,—one culture from seed ripened under normal conditions and another from seed of the second harvest developed under conditions of malnutrition. The companion cultures were grown side by side in the hot-house and in the field and the seed germination was forced after the following method: The seeds after soaking in water for 24 hours, were subjected to alternate exhaust and pressure up to 45 pounds in a metal case, 4 times in 24 hours, and were then placed on pads of wet filter-paper in Petri dishes. The seedlings were set out in pans and the residue of sterile seed-like structures saved to determine the percentage of germination. Germination following this method was found to be complete for all viable seeds.²

² For details concerning the method of forcing *Oenothera* seeds to complete germination, see DE VRIES (1915), and DAVIS (1915, 1918).

TABLE 1

List of F₁ plants from which second harvests of selfed seed were gathered following conditions of malnutrition induced by removing from the plants all leaves and all side branches.

F ₁ PLANT	CROSS	SECOND SELFING	NUMBER OF FLOWERS SELFED	ALL LEAVES AND ALL SIDE BRANCHES REMOVED	PLANT DEAD	SECOND HARVEST
19.58, I-2	L × b	Aug. 16	4	Aug. 18	Sept. 6	{ 2 small shrunken capsules
19.58, I-3	" "	" 12-16	4	" 18	" 6	{ 4 medium-sized shrunken capsules
19.58, III-3	" "	" 12-15	7	" 17	" 13	{ 2 medium-sized capsules
19.58, VI-3	" "	" 12-16	4	" 18	" 6	{ 3 medium-sized shrunken capsules
19.58, VII-3	" "	" 14-16	6	" 18	" 6	{ 3 small shrunken capsules
19.59, I-1	b × L	" 12-14	8	" 16	" 6	{ 3 medium-sized shrunken capsules
19.59, I-3	" "	" 12-15	6	" 17	" 13	{ 2 medium-sized capsules
19.59, II-1	" "	" 12-16	6	" 18	" 6	{ 5 medium-sized shrunken capsules
19.59, IV-1	" "	" 12-16	7	" 18	" 6	{ 2 small shrunken capsules
19.59, IV-2	" "	" 12-15	7	" 17	" 13	{ 1 large and 3 medium-sized capsules
19.59, V-1	" "	" 12-14	8	" 16	" 13	{ 1 large and 4 small capsules
19.59, V-2	" "	" 12-15	7	" 17	" 6	{ 3 large shrunken capsules
19.59, VII-2	" "	" 12-15	10	" 17	" 6	{ 3 large shrunken capsules

The results of the F_2 generations from the reciprocal F_1 hybrids are given in tables 2 and 3 with the more interesting cultures placed first in the tables. A column headed "remarks" gives the loss by death of seedlings or of plants in the field, data which will make clear the percentages of germination obtained. Seedlings recorded as stumpy constitute a definite class peculiar in that no root develops from the end of the hypocotyl and although the seedling becomes green it can live but a few days. The plants other than *brevistylis* and *Lamarckiana* are counted with *Lamarckiana* in determining the proportions of *brevistylis* in the culture as a whole. This means that the mutants such as *nanella*, *oblonga* and *scintillans* have never presented *brevistylis* characters except for one case of a *nanella-brevistylis* (table 3, culture 20.76) which will be described later.

Table 2 gives the data in cultures from selfed seed of 5 F_1 plants of *Lamarckiana* $\times$ *brevistylis*. The cultures are arranged in pairs the results of the experiments (even numbers) following the data from seed ripened under favorable conditions. Cultures 20.53 and 20.54 show a very sharp contrast between normal expectations and results from the experiment; the percentage of germination falls from 30.7 to 7.8, the ratio of *brevistylis* falls from 1:2.81 to 1:10.5, and there is a very large number of stumpy seedlings as the result of the experiment. Cultures 20.55 and 20.56 illustrate a similar correlation of low percentage of germination with a lower proportion of *brevistylis*. The number of plants in the experimental cultures 20.52 and 20.58 are small but there were no *brevistylis* present and both cultures show a low percentage of germination and proportionately large numbers of stumpy seedlings. The culture listed as 20.60 is one of the few cases in which the treatment did not markedly lower the percentage of viable seeds and the ratio of *brevistylis*, 1:4.47, is not much smaller than the ratio, 1:3.79, recorded from the seed produced normally by that plant; also, in 20.60 there was but one stumpy seedling. Taking the set of 10 cultures as a whole it is noteworthy that stumpy seedlings appeared only in the experiments, and in some of these, 20.54 and 20.58, the numbers were large.

Table 3 gives the data in cultures from selfed seed of 8 F_1 plants of *brevistylis* $\times$ *Lamarckiana*. As in table 2 they are arranged in pairs so that the results of the experiments may be readily compared with the data from normally ripened seed. Cultures 20.67 and 20.68 show the sharpest contrasts in all of my results between the experimental and normal conditions; the percentage of germination was lowered from 27.7 to 6.3, the ratio of *brevistylis* fell from 1:2.83 to 1:17, and there were 6 stumpy seedlings from the experiment. Cultures 20.65 and 20.66 show the same trend and

TABLE 2

Cultures in the F_2 from selfed seed of 5 F_1 plants of *Lamarckiana* $\times$ *brevistylis*. The cultures are grouped in pairs (20.51 and 20.52, 20.53 and 20.54, etc.), the odd numbers from seed developed normally, the even numbers from seed produced in the latter half of the season under the experimental conditions of malnutrition described in the text and summarized in table 1.

CULTURE	F_1 PARENT PLANT	SEEDS SOWN	PERCENT- AGE OF GERMINA- TION	NUMBER OF BREV- STYLIS	NUMBER OF LA- MARCKIANA	OTHER PLANTS IN THE CULTURE	RATIO OF BREVISTYLIS TO LA- MARCKIANA AND OTHER PLANTS	REMARKS
20.53	19.58, I-3	417 1 large capsule	30.7	32	88	1 <i>nanella</i> 1 round-leaved dwarf rosette	1: 2.81	5 seedlings died 1 plant died in the field
20.54 Experiment	" "	563 4 medium-sized shrunken capsules	7.8	2	20	1 <i>nanella</i>	1:10.5	18 stumpy seedlings which died 3 other seedlings died
20.55	19.58, VI-3	454 2 medium-sized cap- sules	27.5	37	86	1 <i>oblonga</i>	1: 2.35	1 plant died in the fie'd
20.56 Experiment	" "	374 3 medium-sized shrunken capsules	3.7	3	11		1: 3.66	
20.51	19.58, I-2	406 2 medium-sized cap- sules	24.9	26	63	3 <i>nanella</i> 1 narrow-leaved dwarf rosette	1: 2.58	7 seedlings died 1 plant died in the field
20.52 Experiment	" "	145 2 small shrunken cap- sules	7.6	0	3	1 <i>nanella</i>	0:4	6 stumpy seedlings which died 1 other seedling died

TABLE 2 (continued)

20.57	19.58, VII-3	420 2 medium-sized capsules	29.5	32	88	2 <i>scintillans</i>	1: 2.81	2 seedlings died
20.58 Experiment	" "	256 3 small shrunken capsules	5.5	0	4		0: 4	10 stumpy seedlings which died
20.59	19.58, III-3	484 1 large capsule	29.3	29	109	1 <i>oblouga</i>	1: 3.79	2 seedlings died 1 plant died in the field
20.60 Experiment	" "	395 2 medium-sized capsules	26.8	19	84	1 <i>scintillans</i>	1: 4.47	1 stumpy seedling which died 1 other seedling died

the experiment in this case is of especial interest because of its large proportion of stumpy seedlings in contrast to the complete absence of these abortions from seed ripened under normal conditions. Culture 20.70 illustrates the very exceptional case in which the percentage of germination in the experiment is higher than that of the normal, 20.69, although the ratio of *brevistylis* was markedly lower, suggesting that there may be other factors in the experiment beside the malnutrition of the developing seeds. Then follow a group of cultures (20.75 and 20.76, 20.73 and 20.74, 20.61 and 20.62, 20.71 and 20.72) in which the percentage of germination and the ratio of *brevistylis* are consistently lower in the experiments although the contrast is not so marked as in some other pairs; stumpy seedlings were present in all of the experiments, the proportions being very large in 20.76 and 20.62. Culture 20.76 gave the only instance in all of my cultures (totaling about 5000 plants during three seasons) of the union of *brevistylis* characters with those of a mutant; in this case the association was with the characters of *nanella* giving a *nanella-brevistylis*. Cultures 20.63 and 20.64 would be of greater interest if the experiment had supplied more than the single plant which matured; the treatment in this case was so severe that from 7 flowers pollinated in the experiment only 2 small shrunken capsules were ripened and the 175 seed-like structures gave only 8 seedlings of which 5 were stumpy and 2 died in the seed pans. In this set of 16 cultures it is again important to observe that the stumpy seedlings were developed almost wholly by the experiments.

Considering the combined data as presented in tables 2 and 3, a correlation of three principles may be noted. First, in each of the 13 pairs of cultures the ratio of *brevistylis* to other plants is less in the experiment than in the culture from seed ripened under normal conditions. Second, for each pair the percentage of germination is lower in the experiment with the single exception of culture 20.70 in table 3. Third, the large class of stumpy seedlings (those in which a root fails to develop from the hypocotyl) is found almost wholly in the experiments. This is the evidence for the writer's view that malnutrition of developing seeds in the F_1 generations of this material lowers the ratio of *brevistylis* segregates in the F_2 and in double reciprocals by increasing the mortality of *brevistylis* zygotes or embryos. The fact of the higher death rate is clearly shown in the lower percentage of viable seed following the experiments and the large class of abortive seedlings called "stumpy." The hypothesis holds that through this increased mortality the *brevistylis* class suffers proportionally to a greater degree than does the class of *Lamarckiana*.

That the class of *Lamarckiana* suffers with *brevistylis* under the conditions of the experiment is brought out clearly in table 4. There is given in the last two columns of this table for the experiment in comparison with normal conditions the percentages of *Lamarckiana* and *brevistylis* to the number of seeds sown. In all cases (except for the pair 20.69 and 20.70) the percentage of *Lamarckiana* declines sharply in the experiment, but in all cases the percentage of *brevistylis* falls to a much greater degree and consequently there is the final result in the experiment of a smaller ratio of *brevistylis* to *Lamarckiana*. Thus in the pair 20.53 and 20.54 the percentage of *Lamarckiana* in the experiment fell from 21.1 to 3.5 or to about one-sixth of the normal, but the percentage of *brevistylis* in the experiment fell from 7.7 to 0.3 which is one-twenty-fifth of the normal.

HERIBERT-NILSSON (1920) has recently shown that tubes from different genetic types of pollen may grow down the long styles of *Oenothera* at different rates, making possible greater proportionate numbers of fertilization by those pollen tubes that reach the ovaries first. He offers plausible explanations of some peculiar departures from expectations in segregation on this hypothesis of an elimination of gametes through the slower growth of their pollen tubes. This factor, even if present in the *brevistylis*-*Lamarckiana* crosses, would not account for the results of the experiments here described because, as shown in table 1, the pollinations of the selfings were made at least two days before the plants were stripped of leaves and branches. By this time the flowers had withered and fallen from the plants so that relations between pollen-tube and style could not have been disturbed by the conditions of the experiment.

A considerable range of variation in the ratio of *brevistylis* to *Lamarckiana* in segregation under normal conditions is shown in tables 2 and 3 of this paper and was noted in my earlier paper of 1918. Thus when expectations called for a 1:3 ratio there has been a ratio of *brevistylis* to *Lamarckiana* as high as 1:2.35 based on a family of 124 plants (table 2, culture 20.55), and as low as 1:3.79 in a family of 139 plants (table 2, culture 20.59). These irregularities in ratios in cultures developed from seeds ripened under favorable conditions and forced to complete germination suggest that other factors in addition to malnutrition of seeds may affect relative proportions of *brevistylis*, but such have not yet been isolated. It would not be surprising to find some differences between *brevistylis* and *Lamarckiana* in the germination of pollen or the rate of pollen-tube growth and some such differential may at times be accentuated by conditions of style or stigma to a point at which marked departures from normal behavior may take place.

TABLE 3

Cultures in the F₂ from selfed seed of 8 F₁ plants of brevistylis × Lamarckiana. The cultures are grouped in pairs (20.61 and 20.62, 20.63 and 20.64, etc.), the odd numbers from seed developed normally, the even numbers from seed produced in the latter half of the season under the experimental conditions of malnutrition described in the text and summarized in table 1.

CULTURE	F ₁ PARENT PLANT	SEEDS SOWN	PERCENT-AGE OF GERMINATION	NUMBER OF BREVISTYLIS	NUMBER OF LA-MARCKIANA	OTHER PLANTS IN THE CULTURE	RATIO OF BREVISTYLIS TO LA-MARCKIANA AND OTHER PLANTS	REMARKS
20.67	19.59, I-1	343 1 large capsule	27.7	24	66	1 <i>oblonga</i> 1 <i>scutillans</i>	1:2.83	3 seedlings died
20.68 Experiment	" "	379 3 medium-sized shrunken capsules	6.3	1	17		1:17	6 stumpy seedlings which died
20.65	19.59, V-2	497 2 large capsules	36.2	41	121	1 <i>lata</i> 2 <i>oblonga</i> 2 <i>scutillans</i>	1:3.07	2 seedlings died 1 plant died in the field
20.66 Experiment	" "	486 3 large shrunken capsules	15.8	5	35	1 <i>nanella</i> 1 dwarf rosette	1:7.4	31 stumpy seedlings which died 4 other seedlings died
20.69	19.59, I-3	224 1 medium-sized cap- sule	28.6	14	47	1 <i>oblonga</i>	1:3.43	2 seedlings died
20.70 Experiment	" "	292 2 medium-sized cap- sules	36.3	16	82	3 <i>scutillans</i> 1 broad-leaved dwarf rosette 1 narrow-leaved dwarf rosette	1:5.44	3 seedlings died

TABLE 3 (continued)

20.75	19.59, VII-2	486 2 large capsules	23.2	26	82	2 <i>scintillans</i>	1:3.23	2 stumpy seedlings which died 1 plant died in the field
20.76 Experiment	" "	558 3 large shrunken capsules	17.0	11 1 <i>nanella-bre-</i> <i>vistylis</i>	58		1:4.83	18 stumpy seedlings which died 2 other seedlings died
20.73	19.59, V-1	401 1 large capsule	28.7	26	86	1 <i>scintillans</i>	1:3.35	2 seedlings died
20.74 Experiment	" "	248 1 large capsule	21.8	8	37	1 <i>oblonga</i>	1:4.75	5 stumpy seedlings which died 3 other seedlings died
20.61	19.59, II-1	272 1 medium-sized capsule	23.5	15	47	1 narrow-leaved dwarf	1:3.2	1 seedling died
20.62 Experiment	" "	544 5 medium-sized shrunken capsules	11.8	7	29		1:4.14	23 stumpy seedlings which died 5 other seedlings died
20.71	19.59, IV-2	573 1 large capsule	35.2	49	144	2 <i>nanella</i> 1 <i>scintillans</i>	1:3	6 seedlings died
20.72 Experiment	" "	295 1 large capsule	28.8	16	62	1 <i>oblonga</i> 3 <i>scintillans</i>	1:4.12	3 stumpy seedlings which died
20.63	19.59, IV-1	311 1 large capsule	26.3	22	63		1:2.86	1 stumpy seedling which died 1 plant died in the field
20.64 Experiment	" "	175 2 small shrunken capsules	4.6	0	1		0:1	5 stumpy seedlings which died 2 other seedlings died

TABLE 4

The percentages of Lamarckiana and brevistylis to the number of seeds sown. A comparison of the experiments through which seeds were ripened under conditions of malnutrition with the results from seeds ripened under normal conditions.

CULTURE	CROSS	NUMBER OF SEEDS SOWN	NUMBER OF LAMARCKIANA	NUMBER OF BREVISTYLIS	PERCENTAGE OF LAMARCKIANA TO SEEDS SOWN	PERCENTAGE OF BREVISTYLIS TO SEEDS SOWN
20.51	L × b	406	63	26	15.5	6.4
20.52	" "	145	3	0	2.1	0
Experiment						
20.53	" "	417	88	32	21.1	7.7
20.54	" "	563	20	2	3.5	0.3
Experiment						
20.55	" "	454	86	37	18.9	8.1
20.56	" "	374	11	3	2.9	0.8
Experiment						
20.57	" "	420	88	32	20.9	7.6
20.58	" "	256	4	0	1.6	0
Experiment						
20.59	" "	484	109	29	22.5	5.9
20.60	" "	395	84	19	21.3	4.8
Experiment						
20.61	b × L	272	47	15	17.3	5.5
20.62	" "	544	29	7	5.3	1.3
Experiment						
20.63	" "	311	63	22	20.2	7.0
20.64	" "	175	1	0	0.6	0
Experiment						
20.65	" "	497	121	41	24.3	8.2
20.66	" "	486	35	5	7.2	1.0
Experiment						
20.67	" "	343	66	24	19.2	6.9
20.68	" "	379	17	1	4.5	0.3
Experiment						
20.69	" "	224	47	14	20.9	6.2
20.70	" "	292	82	16	28.1	5.5
Experiment						
20.71	" "	573	144	49	25.1	8.5
20.72	" "	295	62	16	21.0	5.4
Experiment						
20.73	" "	401	86	26	21.4	6.5
20.74	" "	248	37	8	14.9	3.2
Experiment						
20.75	" "	486	82	26	16.9	5.3
20.76	" "	558	58	12	10.3	2.1
Experiment						

There are listed for these cultures in tables 2 and 3 the variants which appeared. These were readily identified as mutants described by DE VRIES except possibly the type that is given as *scintillans*. This form has most of the characters in the description of *scintillans* and its low seed production indicates the probability of its being triploid in chromosome count, but it has not been tested in this respect. The cultures from the experiments gave a larger proportion of mutants than were present in the normal cultures and about the same variety. Thus there were 15 mutants in a total of 546 plants from the experiments or 2.7 percent, while the normal cultures totaling 1488 plants gave 25 mutants or 1.7 percent. Apparently the mutants did not suffer from the treatment of malnutrition proportionately more than did *Lamarckiana* and *brevistylis*.

SUMMARY

1. Harvests of seed from selfed plants in the F_1 generation of reciprocal crosses between *brevistylis* and *Lamarckiana* were collected by the middle of August; these gave F_2 generations from seed ripened under normal conditions. Following the collection of the first harvests the F_1 plants were again selfed and then all of the leaves and side branches were removed so that second harvests of seed were ripened under experimental conditions of malnutrition; these gave F_2 generations from seed ripened under conditions of malnutrition (table 1). The F_2 cultures then grouped themselves in pairs, a normal and an experimental culture having the same F_1 parent plant. All cultures were grown from seed forced to complete germination.

2. The experimental cultures (from seed developed under condition of malnutrition) showed uniformly a smaller percentage of *brevistylis* segregates, and there were such extreme ratios as 1:17, 1:10.5, 1:7.4, 1:5.4, etc., when the ratios in the normal cultures were close to 1:3. These lower ratios of *brevistylis* in the experimental cultures following malnutrition of seed were consistently correlated with lower percentages of germination (one exception), and with the presence in the experimental cultures of large numbers of abortive seedlings, called "stumpy" because a root failed to develop from the tip of the hypocotyl. This is the evidence for the writer's view that malnutrition of developing seeds in the F_1 generation of this material lowers the ratio of *brevistylis* segregates in the F_2 by increasing the mortality of *brevistylis* zygotes or embryos.

3. Since the leaves and side branches were removed from the F_1 plants after the flowers, following the second pollination, had withered and fallen, relations of pollen-tube to style could not have been a factor in determining the lower ratio of *brevistylis* to *Lamarckiana* obtained in the experiments.

4. The class of *Lamarckiana*, as shown in table 4, also suffered with *brevistylis* under the conditions of malnutrition, but in all pairs of cultures the percentage of *brevistylis* fell to a much greater degree and consequently there resulted in the experiments always smaller ratios of *brevistylis* to *Lamarckiana* than in normal cultures.

5. Mutants appeared in both sets of cultures with a slightly larger percentage in the experiments indicating at least that they did not suffer from the treatment of malnutrition proportionately more than did *Lamarckiana* and *brevistylis*.

6. A plant of *nanella* appeared with *brevistylis* characters giving the combination *nanella brevistylis*. This is the only observed instance in cultures of three seasons, totalling about 5000 plants, of the union of *brevistylis* characters with those of a mutant.

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