HYBRIDS OF *OENOTHERA BIENNIS* AND *OENOTHERA FRANCISCANA* IN THE FIRST AND SECOND GENERATIONS¹

BRADLEY MOORE DAVIS

University of Pennsylvania, Philadelphia

[Received December 18, 1915]

TABLE OF CONTENTS

	PAGE
INTRODUCTION	197
Methods of obtaining complete germination of seeds.....	201
A comparison of the parent species, <i>Oenothera biennis</i> Linnaeus and <i>Oe. Franciscana</i> Bartlett	203
Reciprocal crosses between <i>Oenothera biennis</i> and <i>Oe. Franciscana</i>	212
Hybrids of the cross <i>Franciscana</i> E $\times$ <i>biennis</i> in the second generation, complete germination of the seeds,—culture 15.37	216
Hybrids of the cross <i>biennis</i> $\times$ <i>Franciscana</i> E in the second generation, after complete germination of the seeds,—culture 15.38.....	223
Hybrids of the cross <i>Franciscana</i> B $\times$ <i>biennis</i> in the second generation, from seeds germinated in the earth.....	228
Hybrids of the cross <i>biennis</i> $\times$ <i>Franciscana</i> B in the second generation, from seeds germinated in the earth	243
DISCUSSION AND SUMMARY	243
LITERATURE CITED	250

INTRODUCTION

This paper will give the results of a study involving two species of *Oenothera* and their hybrids in the first and second generations. The species, *Oenothera biennis* Linnaeus and *Oe. Franciscana* Bartlett, are sharply contrasted in a color character as well as in characters of form and size, and consequently offer favorable material to test the problem of whether or not a process of protoplasmic differentiation at the end of the F₁ generation leads to the formation of diverse types of gametes, making possible a segregation of characters and the consequent establishment of distinct classes in the F₂ generation. Such a behavior, if clearly evident, would naturally point towards a Mendelian interpretation of the results, if it be admitted that the chief tenet of Mendelism is the principle of segregation following an F₁ generation.

¹ Genetical studies on *Oenothera*—VII.

The observations on certain of the cultures in the F_2 have an especial interest in being, so far as I know, the first cultures of *Oenothera* grown, in which the germination of seeds has been experimentally forced to a finish, giving cultures which at the beginning contained the progeny of all viable seeds. Thus a method is introduced in genetical work among *oenotheras* which the writer believes to be absolutely essential to accurate and trustworthy results in this group of plants. The evidence is clear that large proportions of *Oenothera* seeds sprout in the earth only after many weeks or even months and that this habit of delayed germination must have given in many of the cultures described in the *Oenothera* literature hardly more than glimpses of the genetical possibilities. We cannot feel certain that the records of any cultures of *Oenothera* so far reported are complete for their possible progeny, and consequently the ratios of classes described in breeding experiments and the percentages of "mutants" calculated cannot be accepted as final in exact genetical work. We are not in a position even to guess what may be the changes of front when exact data become available.

The views of DE VRIES have been in general strongly against Mendelian interpretations of his results with the exception of the behavior of *Oenothera brevistylis* which appears to act as a recessive in crosses with *Lamarckiana* and other species. Recently, however, DE VRIES (1915 c) reports the isolation of certain forms of *Oe. gigas* which, when selfed, gave about 18 percent dwarfs, about 25 percent pure *gigas*, and about 57 percent of *gigas*-like "mutant hybrids" similar to the parent plants and which, when selfed, again threw about 21 percent of dwarfs. These impure *gigas* forms are regarded by DE VRIES as being heterozygous for a dwarf factor and consequently exhibiting in their breeding the simple Mendelian ratio of a monohybrid.

Other investigators have in recent years with varying emphasis expressed their doubts as to the applicability of Mendelian principles to various features of *Oenothera* genetics. Thus GATES (1914, 1915 a), largely on studies of crosses between *Oenothera rubricalyx* and *Oe. grandiflora*, came strongly to the conclusion that the "mutants" of *Oenothera* cannot be explained as the appearance of types thrown out by the Mendelian behavior of hybrid material. A recent paper (GATES 1915 b), nevertheless, argues for Mendelian interpretations of certain results. In all of the work of GATES we can have no confidence that his data truly express the genetical situations since the cultures were frequently small and all were apparently grown without consideration of the probability of extensive delayed germination and seed-sterility, and

cannot be expected to give an accurate record of the conditions. SHULL (1914) in a paper on *rubricalyx* in crosses with *Lamarckiana* and *rubrinervis* announces his belief that "a hereditary mechanism must exist in *Oenothera* fundamentally different from that which distributes the Mendelian unit-characters." SHULL's cultures also were grown from seed sown in the earth and are without data on delayed germination and seed-sterility; they also are likely imperfectly to represent the true genetical situation. The work with *rubricalyx* is at present much clouded by the discussion between GATES and SHULL over the origin and genetical constitution of this remarkable plant. BARTLETT (1915 a, b, c, d) in a series of papers has given us the most impressive evidence in recent years for mutation in *Oenothera*, together with arguments against its interpretation as Mendelian phenomena, but the writer (DAVIS 1915 b, c) has already expressed his belief that such studies can only have full value when the material is most thoroughly tested for its genetical purity and when germinations in the cultures are proved to be complete. There is evidence of order in BARTLETT's results, to me in the main very suggestive of Mendelian behavior.

In a paper on hybrids between an American "*Oenothera biennis*" and *Oe. grandiflora* the writer (DAVIS 1912, 1913 a) pointed out what then seemed to him difficulties in interpreting conditions exhibited by the F_2 in accordance with a strict Mendelian conception of the stability and conservation of factors. It was a tentative position of which EAST (1912) has presented pertinent criticism and to which I now attach no importance since there is every reason to believe that my methods of culture at that time, in common with those of all other workers on *oenotheras*, did not give exact genetical data because the seed-germination was undoubtedly incomplete and also for the reason that the parent stocks had not passed sufficiently critical tests for their genetic purity. I have recently discussed the tests that must be applied to a species of *Oenothera* to determine whether or not it is genetically pure (DAVIS 1915 b) and will only emphasize my belief that no type has as yet satisfied such an examination. Consequently we have at present in the *oenotheras* no standard material of genetic purity with which forms under suspicion may be confidently mated to determine by cross-breeding the uniformity of their viable gametes. Until such material is discovered we shall be working largely in the dark in our attempts to analyse the genotypic constitution of *oenotheras*.

Two writers on *Oenothera* genetics, HERIBERT-NILSSON and RENNER, have taken a clear Mendelian attitude in the interpretation of their re-

sults. HERIBERT-NILSSON (1912, 1915) in lengthy papers reports studies on a race of *Lamarckiana* from Sweden. He holds the view, with which I am in complete sympathy, that *Lamarckiana* is an impure or polymorphic species, of hybrid constitution. From this standpoint he develops the theory that its "mutants", which he lists as "combinations", are the result of various assemblings of Mendelian factors following the processes of segregation during the reduction divisions in the parent forms. It is a promising line of study which HERIBERT-NILSSON has followed with persistence. However, because of his methods of sowing seeds in the earth without proper check on the probabilities of incomplete germination we cannot be certain that he has obtained full results in the data which he has gathered, either as to the forms themselves or as to their ratios in the cultures.

We are indebted to RENNER (1914) for the first critical consideration of seed-sterility in *Oenothera* and its possible bearing on the genetical constitutions of forms whose breeding habits are peculiar. He is the first investigator to suggest that seed-sterility in this group may mask a behavior essentially Mendelian in its character. This attitude is well illustrated by his suggestive hypothesis on the genotype of *Lamarckiana*. Since *Lamarckiana* when crossed with *biennis* and certain other species gives the twin hybrids *laeta* and *velutina* it may be assumed to develop two types of gametes which function, and these may be called the *laeta* and *velutina* types. *Lamarckiana* self-pollinated might then give zygotes in the proportions of 1 pure *laeta*: 2 *laeta-velutina*: 1 pure *velutina*. Now more than one half of the seeds of *Lamarckiana* fail to develop normal embryos and RENNER believes that in these sterile seeds are represented zygotes homozygous respectively for the *laeta* and *velutina* factors. The fertile seeds are believed by RENNER to develop from the heterozygotes with *laeta* and *velutina* factors acting together and this combination is held to give the characters of *Lamarckiana*. *Oenothera Lamarckiana* is thus considered by RENNER to be an impure or heterozygous species, breeding true because of the mortality of such zygotes as carry the factors for *laeta* and *velutina* in homozygous conditions. The hypothesis offers a simple Mendelian explanation of the behavior of *Lamarckiana* in producing twin hybrids, and the facts of seed-sterility support the view of its genetic impurity.

The studies of RENNER on seed-sterility are certain to have a profound effect on future investigations in *Oenothera* genetics. DE VRIES (1915 a, b) recognized their importance at once by working out an experimental method by which rapid germination may be obtained through forcing

water into wet seeds by increased air pressure, thus readily obtaining the percentages of germination. Last winter I also took up the problem and tested the seed-fertility of some fifty species, races or hybrids, and I have already published the results of the study for a number of hybrids between *biennis* and *muricata* (DAVIS 1915 b). The immediate result of this understanding of the importance of seed-sterility and delayed germination is likely to be the adoption in the future of methods whereby the germination of seeds will be brought to completion and the residue of sterile seed-like structures preserved for examination.

METHODS OF OBTAINING COMPLETE GERMINATION OF SEEDS

Certain F_2 generations reported in this paper were grown in the past summer (1915), following an experimental method that gave a complete germination of the viable seeds, as proved by the examination of the sterile seed-like structures.

The method has been described in full (DAVIS 1915 a) and will be only briefly outlined at this time. The seeds were germinated on pads of wet filter paper in Petri dishes kept in a warm portion of a hot house. The sprouted seeds with radicles 3 mm or more in length were then set in sterilized soil in square seed-pans and were arranged and properly spaced in definite rows. Thus the seedlings were counted as planted and the death of any became evident. The young rosettes were later transferred from the seed-pans to small pots where they generally became $\frac{1}{4}$ to $\frac{1}{3}$ full grown before they were set out in the garden. The residue of sterile seed-like structures left in the Petri dish was then examined, after germination appeared to be complete, to make sure that no viable seeds remained. The examination is easily made by pressing the structures, now softened by water, between the tips of a strong pair of forceps; any embryos present are at once squeezed out and may be readily examined. The sterile seeds were conveniently arranged, as illustrated in figure 13, and then fastened to the piece of filter paper by flooding the seeds with a solution of shellac in alcohol, thus preserving a record of the residue.

This method is, I think, open to an important improvement which will be tested in future cultures. Following the suggestion of DE VRIES (1915 a, b) I shall subject the thoroughly wet seeds to increased air pressure before placing them in the Petri dishes. This may be readily accomplished by placing test tubes, containing the seeds immersed in water, within a metal conceptacle that may be closed air-tight and into which air may be forced by an automobile pump to a pressure of several atmo-

spheres indicated by a gauge. By thus forcing water into the seeds DE VRIES claims that germination will take place immediately and there should not be a long wait in the Petri dishes before germination is complete.

This paper will also describe certain cultures grown in 1914 after the old methods of sowing seeds in the earth. Following my practice of recent years I counted the seed-like structures that entered the seed-pans and in this way obtained a fair idea of the proportions of seeds which germinated after being in the pans for 8-10 weeks. But it has become evident that delayed germinations under such conditions make impossible accurate genetical work since seeds sown in earth are obviously lost for

FIGURE 1.—Rosette of *Oenothera biennis*, nearly mature, showing the close cluster of broadly-elliptical, crinkled leaves. Contrast with figure 2, *Franciscana*.

further inquiry as to the fact of their viability; a proportion of seedlings appear, but, as for the residue, that cannot be examined.

I have laid emphasis on the necessity of developing methods of experimental germination of seeds in genetical work in *Oenothera* for reasons outlined in the introduction. The practice of sowing seeds directly in the earth must always be open to sharp criticism because of the conditions of seed-sterility and the habit of delayed germination prevalent in this group

of plants. It may again be noted that none of the published research in *Oenothera* genetics has as yet satisfied the conditions of the problems and that none of the results so far as they report ratios of classes, segregates, or "mutants," can be regarded as fully trustworthy.

A COMPARISON OF THE PARENT SPECIES, *OENOTHERA BIENNIS* LINNAEUS AND *OE. FRANCISCANA* BARTLETT

The race of *Oenothera biennis* employed was that described in my paper "Some reciprocal crosses of *Oenothera*" (DAVIS 1914). It is the plant made familiar to us by the studies of DE VRIES and STOMPS and

FIGURE 2.—Rosette of *Oenothera Franciscana* B, nearly mature, showing the close cluster of narrower leaves, less strongly crinkled. Contrast with figure 1, *biennis*.

represents a type wide-spread on the sand dunes of Holland and also present in England and in other European countries. BARTLETT (1913) has given strong reasons why it should be regarded as the form known to Linnaeus as *Oenothera biennis* and consequently to be regarded as the type form of the species. This Dutch plant in garden cultivation has proved to be remarkably stable. However, STOMPS (1914) in cultures

of selfed lines from a single plant collected in 1905, discovered that the Dutch *biennis* throws occasional marked variants ("mutants"). DE VRIES (1915 a) in large cultures totaling 8500 plants from the same lines established by STOMPS, found that the three variants appeared in the following percentages, (1) *biennis semi-gigas* (21 chromosomes and self-sterile) about 0.05 percent, (2) *biennis nanella* (dwarf) about 0.1 percent, and (3) *biennis sulfurea* (a variety with pale yellow petals) about 0.3 percent. Since the percentages from *Lamarckiana* are for *semi-gigas* 0.3 percent and for *nanella* 1 to 2 percent, it may be noted that with respect to these "mutants" *biennis* appears to be the more stable of the two species. The color variety *biennis sulfurea* is a type of variant new in experimental studies on *oenotheras* and it is interesting to record that a single plant exhibiting its peculiarity appeared in the F_2 of a cross *biennis* $\times$ *Franciscana* B described on page 243 of this paper.

FIGURE 3.—Mature plant of *Oenothera biennis*. Showing long side-branches and a foliage of large, broadly-elliptical leaves. Contrast with figure 4, *Franciscana*.

The species *Oenothera Franciscana* was recently isolated by BARTLETT (1914) from among the oenotheras of the Pacific coast. I have this species now from two sources: (1) *Franciscana* B, the strain which BARTLETT obtained from seed collected at Carmel Beach, Monterey, California, and (2) *Franciscana* E from seed collected by Miss EASTWOOD in San Mateo County, California. Both races have been uniform through three seasons of garden culture of 52, 18 and 16 plants for *Franciscana* B and 140, 18 and 16 plants of *Franciscana* E. Although at present we know the species only from the region of central California, where it appears to be not uncommon, there seems to be no reason why it may not prove to be widely distributed in the western and southwestern United States. I saw last summer in the garden of Professor BARTLETT cultures of two oenotheras clearly in the *Franciscana* alliance, one from the Santa Rita mountains, and the other from the Santa Catalina mountains, in Arizona.

FIGURE 4.—Mature plant of *Oenothera Franciscana* B. Showing shorter side-branches and a foliage of smaller, narrower leaves. Contrast with figure 3, *biennis*.

There are no essential taxonomic differences between these two races of *Franciscana* from California except that *Franciscana* E appears to be somewhat more vigorous. Nevertheless, there have been important differences between the F_2 progenies in crosses with *biennis*, and *Franciscana* B has given a more varied assortment of forms than *Franciscana* E in spite of the fact that the F_2 cultures of the crosses with *Franciscana* E were from seeds in which germination was experimentally established to be complete.

My first crosses were made between *biennis* and *Franciscana* B with the assistance of BARTLETT who had called my attention to the characters of *Franciscana* as likely in combination with those of *biennis* to give hybrids resembling the *Lamarckiana* of DE VRIES. We made in 1912 reciprocal crosses between a plant of *Franciscana* B grown in Washington and a plant of *biennis* in Philadelphia, exchanging pollen by post, and the hybrids from these two crosses furnished the material for part of this paper. Since then I have made reciprocal crosses between *biennis* and *Franciscana* E and carried hybrids through the second generation.

To bring the contrasting characters of *Oenothera biennis* and *Oc. Franciscana* clearly before the reader the two species will first be described in parallel columns. The descriptions are of plants started under glass during the winter and in the spring set out as rosettes in the experimental garden. By this treatment the biennial habit may be broken and the plants brought to maturity during the summer.

Oenothera biennis Linnaeus
 Cotyledons. 6—7 mm long, broad at the base and short-petioled.
 Young rosettes. A close cluster of broadly-elliptical or ovate leaves, short-petioled.
 Mature rosettes. 3.5—4 dm broad. A close cluster of broadly-elliptical leaves tapering to relatively short petioles, crinkled (figure 1), mid-veins reddish. Central shoot generally appearing from the rosette before the side branches.

Oenothera Franciscana Bartlett
 Cotyledons. 8—9 mm long, broadly elliptical and long-petioled.
 Young rosettes. A close cluster of elliptical leaves, narrower and long-petioled.
 Mature rosettes. 3.5—4.5 dm broad. A close cluster of narrower leaves, somewhat spatulate and longer petioled, less strongly crinkled (figure 2), mid-veins reddish. Side branches from the rosette frequently appearing before the central shoot.

Mature plants. About 1 m high. Numerous long side-branches from the rosette, in length almost equal to the central shoot (figure 3). Papillae at the base of long hairs follow the color of the clear green stems. Green stems with a scant pilose pubescence.

Foliage. Leaves broadly elliptical, a lighter green, thin, large (figures 3 and 5).

Inflorescence. Compact. Bracts $\frac{1}{4}$ — $\frac{1}{2}$ length of mature buds, broad near the base (figure 5).

Mature buds. 5.5—6 cm long. Cone 2—2.3 cm long, slightly 4-angled. Sepals green, less heavily pilose and puberulent. Sepal tips 3—4 mm long, slender. Papillae on ovaries green.

Petals. 2—2.3 cm long, a golden yellow.

Stigma lobes. 4—5 mm long, low, generally about 3 mm below the tips of the anthers. Close pollinated.

Capsules. 2—2.3 cm long, stout, pilose and puberulent.

Pollen. About 50 percent abortive.

Seeds. Almost wholly fertile.

Mature plants. 1—1.2 m high. Numerous long side-branches from the rosette, shorter than the central shoot (figure 4). Papillae at the base of long hairs are bright red over the green stems. A heavy pilose pubescence from the red papillae.

Foliage. Leaves narrow elliptical, a darker green, thicker, relatively small (figures 4 and 6).

Inflorescence. More open. Bracts $\frac{1}{3}$ — $\frac{1}{2}$ length of mature buds, narrower (figure 6).

Mature buds. 8.5—9 cm long. Cone 3.2—3.5 cm long, strongly 4-angled. Sepals streaked with red, heavily pilose and puberulent. Sepal tips 5—6 mm long, thick. Papillae on ovaries red.

Petals. 3—3.5 cm long, a lighter yellow.

Stigma lobes. 4—5 mm long, high, from 5—7 mm above the tips of the anthers. Open pollinated.

Capsules. 2.8—3 cm long, slender, heavily pilose and puberulent.

Pollen. Few abortive grains.

Seeds. About 60 percent fertile.

From the above descriptions and by comparison of the figures it may readily be seen that the two species differ conspicuously in many points of size, form and relation of their parts. The chief contrasting features of morphology concern (1) the form of the rosette leaves (figures 1 and 2), (2) the foliage of the mature plants (figures 3 and 4, 5 and 6), (3) pubescence of the stems, (4) the form of the bracts (figures 5 and 6), (5) the size of the buds, form of the sepal tips and relative density of the pubescence on the sepals, (6) the size of the petals, (7) the relative positions of the stigma lobes with reference to the tips of the anthers, and (8) the form and length of the capsules and density of their pubescence.

With respect to characters of color there is (1) a difference in the shade of yellow in the petals, (2) the presence of red streaks on the sepals of *Franciscana* and a greater amount of red over old portions of the stems, and (3) in *Franciscana* the bright red coloration of papillae over the green stems and ovaries; sepals and papillae in *biennis* are green. Differences in the depth of yellow in the petals proved very difficult to distinguish and I was unable to follow such differences accurately

FIGURE 5.—Inflorescence of *Oenothera biennis*. Showing its compact structure and broad bracts. At the left is a leaf from near the base of the main stem. Contrast with figure 6, *Franciscana*.

through the hybrids. The depth of red in the sepals of the hybrids likewise presented so many degrees of intensity, obviously related to the age of the plant, that it also proved unsatisfactory for a genetical

study. The color of the red papillae on stems and ovaries was, however, always brilliant, so that I never had doubts of its presence in my classification of hybrid material.

Since I have given especial attention to the behavior of red papillae in hybrids of the F_1 and F_2 generations it is important to examine the evidence of the purity of the parents with respect to the presence of this character in *Franciscana* and its absence in *biennis*. DE VRIES and

FIGURE 6.—Inflorescence of *Oenothera Franciscana* B, showing its more open structure and narrower bracts. At the right is a leaf from near the base of the main stem. Contrast with figure 5, *biennis*.

STOMPS in letters to me report that they have never noted red papillae on the stems of *biennis* in their cultures of many thousands of individuals and my cultures derived from seed of DE VRIES have for the past four

years been uniformly green-stemmed. We know less of *Franciscana*, but BARTLETT and I have between us grown several hundred plants and red papillae have always appeared in full and striking coloration.

It will be of interest to think for a moment of these two species, *biennis* and *Franciscana*, with reference to the characters that each may contribute to the synthesis of hybrid types resembling the *Lamarckiana* of DE VRIES'S cultures. The mature rosettes of *Lamarckiana* present leaves in form somewhat between those of *biennis* and *Franciscana* but more crinkled. *Lamarckiana* has the same habit of sending up long side-

FIGURE 7.—Rosette of the F_1 hybrid, 14.37 ac, *Franciscana* E $\times$ *biennis*. Clear evidence of a blend in the form of the leaves with, however, patroclinous tendencies. Compare with figure 1, *biennis*, and contrast with figure 8, *biennis* $\times$ *Franciscana* E.

branches from the rosettes, its leaves are large as in *biennis* although more crinkled, its green stems bear red papillae at the base of long hairs as in *Franciscana*. The inflorescence of *Lamarckiana* combines large buds and large flowers as in *Franciscana* with bracts more like those of *biennis*. Its buds are about the same size as those of *Franciscana* but the cone is somewhat stouter and not so strongly 4-angled; the sepals

are sometimes faintly streaked with red, conspicuously so in *rubrinervis* which has very much the color of *Franciscana*; the sepal tips in form are more like those of *biennis* but longer; the pubescence of the sepals is intermediate between these two species; the ovaries have red papillae as in *Franciscana*. The flowers of some forms of *Lamarckiana* are somewhat larger (petals 4—4.5 cm) than those of *Franciscana*, in other forms (petals 2.5—3 cm) smaller; the petals are a golden yellow as in *biennis*; the position of the stigma lobes in the large-flowered forms, 5—7 cm above the tips of the anthers, is as in *Franciscana*. The cap-

FIGURE 8.—Rosette of F_1 hybrid, 14.38 a, *biennis* $\times$ *Franciscana* E. More strongly patroclinous in the form of the leaves and the lesser degree of their crinkling. Compare with figure 2, *Franciscana*, and contrast with figure 7, *Franciscana* E $\times$ *biennis*.

sules of *Lamarckiana* are similar to those of *biennis* in form and size although more variable, exhibiting a considerable range in different races. Thus we see that *biennis* and *Franciscana* have between them characters which, segregating in pure or somewhat modified forms in the F_2 or later generations, might in some individuals readily present combinations that would give *Lamarckiana*-like types. I believe that this is exactly what

has happened for certain plants in the F_2 and F_3 generation of the cross *Franciscana* B $\times$ *biennis*, but I shall continue the lines further before reporting upon the results in full.

RECIPROCAL CROSSES BETWEEN *OENOTHERA BIENNIS* AND *OE. FRANCISCANA*

In a recent paper (DAVIS 1914, pp. 190-191) I have described reciprocal crosses between *biennis* and *Franciscana* and contrasted their characters in parallel columns. These details need not be presented again and I shall give here only a brief summary of the chief points of distinction together with a set of figures illustrating many of these features.

The mature rosettes of the reciprocal crosses are contrasted in figures 7 and 8. That of *Franciscana* $\times$ *biennis* (figure 7) has large leaves, broadly elliptical or spatulate, and strongly crinkled. Clearly a blend of parental characters (compare figure 7 with figures 1 and 2), there are, however, some patroclinous tendencies, although not conspicuous. The mature rosette of *biennis* $\times$ *Franciscana* (figure 8) presents leaves much

FIGURE 9.—Mature plant of F_1 hybrid, 13.35 ac, *Franciscana* B $\times$ *biennis*. Patroclinous in habit and foliage. Compare with figure 3, *biennis*, and contrast with figure 10, *biennis* $\times$ *Franciscana* B.

less crinkled and narrower than in the reciprocal (figure 7) to which it presents a sharp contrast; it is more strongly patroclinous (compare figure 8 with figure 2).

The mature plants are readily distinguished in habit and foliage. Those of *Franciscana* $\times$ *biennis* (figure 9) show the *biennis* habit of side branches from the rosette almost equal to the central shoot (compare with figure 3); the leaves (figure 11), although in form intermediate between the parents, are relatively broad. The plants of *biennis* $\times$ *Franciscana* (figure 10) resemble *Franciscana* in having side branches from the rosette much shorter than the central shoot (compare with figure 4); the leaves (figure 12) are narrower than in the reciprocal. Thus both crosses in habit and foliage are sharply distinguished and both, inclining towards the pollen parent, show patroclinous tendencies.

FIGURE 10.—Mature plant of F_1 hybrid, 13.36 a, *biennis* $\times$ *Franciscana* B. Patroclinous in habit and foliage. Compare with figure 4, *Franciscana* B, and contrast with figure 9, *Franciscana* B $\times$ *biennis*.

The pubescence of the stem (pilose and puberulent), although similar in kind in the two reciprocals, differs markedly in amount, the hybrids being in this respect also patroclinous. Only in the fact that the papillae over green portions of the stem are bright red in both hybrids do we find agreement and this is a very striking fact which would indicate dominance of this character of *Franciscana* over its absence in *biennis*.

FIGURE 11.—Inflorescence of F_1 hybrid, 14.37 ac, *Franciscana* E $\times$ *biennis*. Patroclinous in the broader bracts and slender sepal tips. At the right is a leaf from near the base of the main stem. Compare with figure 5, *biennis*, and contrast with figure 12, *biennis* $\times$ *Franciscana* E.

The inflorescence and buds of the reciprocal crosses likewise show distinctive features partially shown in figures 11 and 12. In their breadth the bracts incline towards the pollen parents. The buds of *Franciscana* $\times$ *biennis* (figure 11), 6.5—7 cm long, are intermediate in

size between the parents, but in the slender form of the sepal tips and in their less heavy pubescence they resemble more closely *biennis*. The buds of *biennis* $\times$ *Franciscana* (figure 12), also 6.5–7 cm long, resemble *Franciscana* in the strongly 4-angled cone, thick sepal tips and very heavy pubescence. The sepals in both crosses are streaked with red in varying depths of color and the ovaries bear red papillae so that

FIGURE 12.—Inflorescence of F_1 hybrid, 14.38 a, *biennis* $\times$ *Franciscana* E. Patroclinous in the narrower bracts and thick sepal tips. Compare with figure 6, *Franciscana*, and contrast with figure 11, *Franciscana* E $\times$ *biennis*.

again a color character present in *Franciscana* appears to dominate over its absence in *biennis*.

The petals offer points of difference in size and color too small for consideration in view of the variations present even upon the same plant, but the size, 2.1–2.5 cm long, is close to that of *biennis*.

The position of the stigma lobes, near the level of the anther tips, is intermediate between the conditions in close-pollinated *biennis* and open-pollinated *Franciscana*; the lobes, 6—7 mm long, are longer than in either parent.

The capsules of *Franciscana* $\times$ *biennis*, 2—2.8 cm long, are shorter and have a less heavy pubescence than in *biennis* $\times$ *Franciscana*, 2.2—3 cm long. In these respects the reciprocal crosses are also patroclinous, but these characters are intermediate between the parents.

We have noted that there are patroclinous tendencies expressed in a variety of characters of the reciprocal crosses, but I have emphasized the fact (DAVIS 1914) that a *morphological* character appears never to be passed on to the F_1 hybrids exactly as it is found in either parent. Even where the morphological resemblance to one of the parents is strongest I have always found evidence of the influence of the other species. With respect to the red pigmentation of the papillae on the stems and ovaries, and of the red coloration of the sepals the presence of this color character in both reciprocals indicates as noted above the dominance in this respect of *Franciscana* over *biennis*. The hybrids proved to be matroclinous as to the time of appearance of the side branches from the rosettes relative to the growth of the central shoot.

HYBRIDS OF THE CROSS *FRANCISCANA* E $\times$ *BIENNIS* IN THE SECOND GENERATION,
AFTER COMPLETE GERMINATION OF
THE SEEDS,—CULTURE 15.37

An F_1 plant, 14.37 *ac* (figures 7 and 11), representative of the cross *Franciscana* E $\times$ *biennis* furnished the seed for this culture. The contents of 6 capsules were sown on January 30 in a Petri dish. Germination ran for about 8 weeks (until March 29) and gave 513 seedlings. On April 8 the residue was examined and 450 sterile seeds were found to be empty of contents; in addition there were 70 small undeveloped structures together with ovule powder; 19 seeds were found containing small amounts of cellular contents. Thus from a sowing of about 982 (513+450+19) seed-like structures 513 seedlings appeared—a germination of 52 percent.

Observations on delayed germination of seeds sown in earth were made on another culture from the same parent plant 14.37 *ac*. The sowing of 396 seed-like structures from 4 capsules was made on January 8, 1915, in sterilized soil and the seedlings picked off and counted as they appeared. After 8 weeks (March 5) the culture had yielded 86 seedlings,—a germination of 21.7 percent. After 14 weeks (April 15) the

number of seedlings totaled 111,—a germination of 28 percent. With 18 weeks (May 15) the number had risen to 164 seedlings,—a germination of 41 percent. After 25 weeks (July 1) the culture had produced 192 seedlings,—a germination of 48.4 percent. During the summer 2 more seedlings appeared, and in February, 1916, still another. The seed-pan was emptied 16 months after the sowing of January 8, 1915. In all 195 seedlings appeared,—a germination of 49 percent. It required 25 weeks to give results accomplished in the Petri dish before 8 weeks. The worker content to accept the result of 8 weeks in the seed-pan would in this case obtain germinations from less than $\frac{1}{2}$ of the viable seeds. The results point a sharp criticism of the past methods of genetical research among the *oenotheras* indicating how fragmentary may have been the cultures upon which ratios have been figured and conclusions have been based.

There was a high mortality among the 513 seedlings and young plants of this culture in comparison with other cultures from seeds germinated experimentally and I had only 466 rosettes at the time when the culture was set out in the garden. These $\frac{1}{3}$ grown rosettes while in pots fell clearly into groups and were planted as follows:

- 20 rosettes with leaves narrower than in *Franciscana*,
- 204 rosettes *Franciscana*-like,
- 40 rosettes *biennis*-like,
- 54 rosettes intermediate between *biennis* and *Franciscana*,
- 24 rosettes with leaves strongly obtuse-pointed,
- 107 rosettes, dwarf and weak, with sickle-shaped leaves,
- 17 rosettes of weak dwarfs and backward plants which could not be placed in the groups above.

Some of these rosette peculiarities were later found to be correlated with characters of the mature plants and thus gave evidence of a prospective segregation of the culture. As the young rosettes grew to full size in the garden some of them changed their characteristics, thus rendering necessary a modification of the above count made at the time when the plants were set out in the open. These changes affected chiefly the 54 rosettes intermediate between *biennis* and *Franciscana* which generally came to resemble more closely one or the other of the parents.

The culture at maturity presented an assemblage which could be readily analyzed for a few characters but was extremely complex for others. My observations were centered on (1) the mature rosette, (2) the presence or absence of red coloration in the papillae of the stem and

ovaries, (3) the character of the foliage whether *biennis*-like or *Franciscana*-like in form, color and texture, (4) the form of the bud tips, (5) size of the petals, (6) the position of the stigma, whether above or below the tips of the anthers, (7) the type of capsule. The form of rosettes, coloration of papillae, character of foliage, form of bud tips, and type of capsule definitely followed certain principles of correlation. The size of petals and position of stigma followed no fixed law of association that I could discover. In general *biennis*-like plants had small flowers and *Franciscana*-like plants large flowers but there were numerous exceptions to this association and also many plants bore flowers in varying degrees intermediate between the parents.

In the tables that follow I shall designate certain characters as *biennis*-like or *Franciscana*-like, but this will not mean that the parental characters are always exactly duplicated. It will, however, signify resemblances so strong that there could be no hesitation in separating the plants under examination and furthermore the classes were without connecting intermediate forms. These characters generally appeared somewhat modified but there were cases where they were indistinguishable from those of the parent species. The study convinced me that a process of segregation determined the results exhibited by the culture and thereby illustrated the fundamental principles of Mendelian inheritance, i.e., segregation in the F_2 generation.

I shall lay no emphasis on the ratios presented by the results since the data is obviously imperfect for two reasons. First, there was a large mortality, especially among the dwarfs and groups of weaker plants which for the most part died before reaching maturity. Second, in a cross with so small a proportion of viable seeds (52 percent) and where the pollen of both parents is also partially sterile there is open the possibility of the elimination of large numbers and perhaps even classes of segregates the absence of which would very seriously complicate the situation.

The field notes which I took on the culture after the rosettes had reached maturity, at the time of flowering, and later, led to the arrangement presented in the following table. If this is compared with the list of groups as set out in the garden two important changes will be noted. First, the group of 54 rosettes previously listed as intermediate between *biennis* and *Franciscana* has disappeared because most of these plants later took on characters *biennis*-like although some became *Franciscana*-like. Second, a group of plants is noted in which the central shoot developed at once from an evanescent rosette. This group comprised plants

TABLE I

The chief segregates in culture 15.37, the F₂ of Franciscana E × biennis.

Mature rosettes	Papillae on stems and ovaries	Foliage at maturity	Bud tips	Capsules
Leaves narrower than <i>Franciscana</i> 16	Red 9	<i>Franciscana</i> -like in color and texture, but narrower-leaved 9	<i>Franciscana</i> -like 5	<i>Franciscana</i> -like 2
<i>Franciscana</i> -like 182	Red 177	<i>Franciscana</i> -like 177	<i>Franciscana</i> -like 174	<i>Franciscana</i> -like 171
<i>biennis</i> -like 67	Red 63	<i>biennis</i> -like 63	<i>biennis</i> -like 62	<i>biennis</i> -like 62
Central shoot developing at once from an evanescent rosette 35	Red 33	<i>Franciscana</i> -like 29 <i>biennis</i> -like 4	<i>Franciscana</i> -like 25 <i>biennis</i> -like 4	<i>Franciscana</i> -like 19 <i>biennis</i> -like 4
Leaves strongly obtuse-pointed. Rosette evanescent 24	Green 23	<i>Franciscana</i> -like in color and texture, but broader-leaved 23	<i>Franciscana</i> -like 23	<i>Franciscana</i> -like 23
Dwarfs, leaves sickle-shaped 59	Red 39	<i>Franciscana</i> -like 36 <i>biennis</i> -like 3	<i>Franciscana</i> -like 36 <i>biennis</i> -like 3	<i>Franciscana</i> -like 30 <i>biennis</i> -like 3
Other dwarfs which died as rosettes 12				
Totals 395	344	344	332	314

some of which appeared among those previously listed as *Franciscana*-like and *biennis*-like, but also included some previously listed as dwarfs.

Before considering such data as is presented in the table above it will

be well to note how great was the mortality of the culture as a whole after the germination of the seeds, and also the much reduced number of plants upon which observations could be made with respect to the color of papillae, foliage, bud tips, and capsules. It will be remembered that the germination gave 513 seedlings of which only 466 produced rosettes in the hot-house. The number of rosettes that reached maturity in the garden amounted to only 395, the mortality naturally being greatest among the dwarfs although occurring also in other groups. The number of plants that sent up shoots upon which observations could be made respecting the color of papillae and character of foliage was 344, the number of plants that produced buds was 332 and capsules were developed upon 314 plants. Thus of 513 seedlings only 314 completed the life cycle, the remaining 199, either because of weak constitution or through accidental death, failed to reach full maturity. This mortality or failure to reach full maturity does not signify unfavorable garden conditions; it is the expression of phenomena that I have found characteristic of this cross and very general among species hybrids of *Oenothera*. It means that immense numbers of zygotic combinations are constitutionally defective or physiologically weak.

Taking the data as they stand there are, however, some very interesting correlations of characters, the most important of which will be noted in the following paragraphs.

The behavior in the F_2 of the color character of red in the papillae on stems and ovaries furnished an illustration in the culture, of the segregation of a character present in one parent, *Franciscana*, and absent in the other species, *biennis*. The character was dominant in the F_1 generation and consequently in the F_2 there might be expected a class of recessives with green stems and ovaries. Such green plants appeared and were wholly confined to the group with evanescent rosettes the leaves of which were strongly obtuse-pointed. Careful search on these plants failed to disclose any red papillae. All other plants of the culture presented red papillae, even those *biennis*-like in other respects, but the numbers of papillae distributed over the stems and ovaries varied greatly with different individuals. The ratio of green plants (23) to those with red papillae (321) is as 1:14, but in view of the pollen- and seed-sterility together with the high mortality in the culture, I lay no stress on this ratio which some geneticists might hail as significantly close to the 1:15 ratio. It is, however, proper to emphasize the fact that there is here presented a clean-cut segregation, Mendelian in its essential features.

A surprising feature of the culture at maturity was its sharp division

into two groups of plants which, with respect to foliage, bud tips, and capsules, resembled either the *Franciscana* or the *biennis* parent. The distinctions between the groups was so clear that no difficulty was experienced in separating the plants. Those *Franciscana*-like had a foliage of narrower, thicker leaves of a darker shade of green, thicker sepal tips, and longer capsules. Those *biennis*-like presented a foliage of broader leaves more thin in texture and lighter in color, sepal tips slender, and shorter capsules. There was generally a heavier pubescence on plants *Franciscana*-like and they were usually taller than those which resembled *biennis*. A record of the foliage characters at maturity showed 274 plants resembling *Franciscana* and 70 *biennis*-like.

Within the *Franciscana*-like group there was a remarkable range of flower-size and a varied position of the stigma whether high or low, i.e., whether above or below the tips of the anthers. There is presented in table 2 the data for 219 of the 274 plants *Franciscana*-like in foliage, a record imperfect because of the slowness or failure of many dwarfs to flower. Table 2 shows clearly a tendency towards the association within the same flower of large petals and a high position of the stigma, both characters of *Franciscana*. It likewise shows a less definite association of small petals with the low position of the stigma, both characters of *biennis*. Petals intermediate in size between the parents are more likely to have a stigma *Franciscana*-like in position. It is important to note that 52 plants with large flowers and high stigmas also resembled *Franciscana* so strongly in their rosettes, red papillae, foliage, bud tips, and capsules as virtually to constitute a class of *Franciscana*-like segregates. The differences between them and the parent species were small plus and minus expressions of the characters of *Franciscana*. In the

TABLE 2

Position of stigma relative to the size of petals on plants *Franciscana*-like in foliage; culture 15.37, the F_2 of *Franciscana* E $\times$ *biennis*.

Petals	Stigma high (<i>Franciscana</i> -like)	Stigma low (<i>biennis</i> -like)	Stigma intermediate	Totals
Large (<i>Franciscana</i> -like)....	52		2	54
Small (<i>biennis</i> -like).....	11	49	55	115
Intermediate	29	1	8	38
Smaller than <i>biennis</i>		7	5	12
Totals.....	92	57	70	219

group of 12 plants with flowers smaller than *biennis* were 3 individuals with petals 9 mm long and crumpled.

Within the second group, *biennis*-like as to foliage, there was likewise a wide range of flower-size and a varied position of the stigma whether high or low. The data for 64 of the 70 plants of this group is presented in table 3. It will be seen that of the 7 large-flowered plants only one had stigmas in the low position. There was a clear tendency for plants with small flowers to be also *biennis*-like in the position of the stigma. Flowers intermediate in size between the parents showed no marked tendency as to the position of the stigma. In the group of 64 plants there were 33 plants essentially indistinguishable from the *biennis* parent in their rosettes, bud tips, flowers, and capsules as well as in foliage. They would have formed a class of *biennis*-like segregates except for the fact that all had red papillae on their stems and ovaries, although on some plants the number of red papillae was small. The culture thus furnished no illustration of a complete segregation of *biennis* characters since the 23 plants with green papillae were all in a group *Franciscana*-like in foliage, bud tips, and capsules (see table 1).

Finally, the large group of dwarfs should be briefly considered. Almost all of these were recognized when young rosettes by their slower growth and by the fact that their leaves were generally asymmetrical or sickle-shaped, as illustrated in figure 18. Although fully green in color they were vegetatively weak from the beginning. Some died in the hot-house and many others failed to survive the conditions of the garden where a slight degree of drought proved to be fatal. Of the 107 dwarf rosettes with sickle-shaped leaves which were set in the garden only 39 produced shoots. Some of these became fair sized but most of them reached a stature of not more than 2—4 dm, having much the appearance shown in figure 19. Of the 39 plants which reached maturity 36 were *Franciscana*-like in foliage and bud tips, while only 3 presented the aspects of *biennis*. From the fact that some dwarf rosettes produced fair-sized plants and thus largely outgrew their prospective condition, and also because a few dwarfs appeared in groups originally composed of strong rosettes it is possible that their weakness may be the effects of a diseased condition rather than due to a specific genetical constitution. This point must be tested by further experimentation to establish conclusions.

TABLE 3

Position of stigma relative to the size of petals on plants biennis-like in foliage; culture 15.37, the F_2 of *Franciscana* E $\times$ *biennis*.

Petals	Stigma high (<i>Franciscana</i> -like)	Stigma low (<i>biennis</i> -like)	Stigma intermediate	Totals
Large (<i>Franciscana</i> -like)....	5	1	1	7
Small (<i>biennis</i> -like).....	1	34	4	39
Intermediate	5	5	5	15
Smaller than <i>biennis</i>		3		3
Totals.....	11	43	10	64

HYBRIDS OF THE CROSS *BIENNIS* $\times$ *FRANCISCANA* E IN THE SECOND GENERATION, AFTER COMPLETE GERMINATION OF THE SEEDS,—CULTURE 15.38

This culture came from an F_1 plant, 14.38 *a*, (figures 8 and 12) of the cross *biennis* $\times$ *Franciscana* E, the exact reciprocal of the plant, 14.37 *ac*, which gave the F_2 generation described in the previous section. Sowings on January 30 of the contents of 2 capsules in a Petri dish gave after 3½ weeks (February 24) 322 seedlings. No other germinations appearing, the residue (figure 13) was examined on March 10 and found to contain about 240 sterile seeds empty of contents and in addition about 80 small undeveloped structures together with ovule powder (figure 13). Thus from a sowing of about 562 (322 + 240) seed-like structures 322 seedlings developed,—a germination of 57 percent.

Another comparative test of delayed germination of seeds sown in earth was made in a culture from the same parent plant, 14.38 *a*. A sowing of 367 seed-like structures from 2 capsules was made on January 8, 1915, in sterilized soil. After 8 weeks (March 5) 104 seedlings had appeared,—a germination of 28 percent. After 14 weeks (April 15) the culture had yielded 187 seedlings,—a germination of 50.9 percent. With 18 weeks the number of seedlings had risen to 200,—a germination of 54 percent. After 25 weeks (July 1) the culture produced in all 243 seedlings,—a germination of 66 percent. During the summer 3 seedlings appeared and in the autumn 4 more. The pan was emptied 16 months after the sowing of January 8, 1915. In all 250 seedlings were obtained,—a germination of 68 percent, which is somewhat higher than that obtained in the Petri dish (57 percent) and it is of interest to note

that both cultures were from sowings of 2 capsules each, but that in the Petri dish consisted of about 562 while that in the earth was of 367 seed-like structures. The smaller number of seed-like structures in a capsule in this case appeared to result in a higher percentage of viable seeds. As illustrated in the case of the culture previously described a worker content to accept the germination from 8 weeks in the seed-pan would have obtained a germination from less than $\frac{1}{2}$ of the viable seeds. So again, the method of experimental and complete germination of seeds justifies the criticism which I offer of past methods of genetical work among the *oenotheras*.

The mortality among the seedlings of this culture was very much less than in the culture previously described since from 322 seedlings 302

FIGURE 13.—The residue after complete germination in a Petri dish from a sowing of about 562 seed-like structures, culture 15.38, F_2 of *biennis* $\times$ *Franciscana* E. The germination produced 322 seedlings and there was left, as the residue, about 240 empty structures the size of seeds and about 80 structures of smaller dimensions which graded into ovule powder.

rosettes developed in the hot-house. The $\frac{1}{3}$ -grown rosettes were planted in the following groups:

- 16 rosettes with leaves narrower than in *Franciscana*,
- 236 rosettes *Franciscana*-like in form but frequently broader-leaved,
- 44 rosettes dwarf and weak, with sickle-shaped leaves,
- 6 rosettes of weak dwarfs which could not be placed in the groups above.

There was the usual modification of characters in some of the rosettes as they came to maturity, rendering necessary a reclassification and modification of the above counts made at the time when the plants were set in the ground. A very large mortality among the rosettes lowered the number which reached maturity from 302 to 247, and of the latter only 208 survived to send up shoots. The mortality was greatest among the dwarfs but also affected rosettes that were *Franciscana*-like.

With the appearance of the shoots the resemblance of the culture as a whole to *Franciscana* became more marked. The foliage at maturity was wholly of the *Franciscana* type in its darker green color, thicker texture and in leaf form although the leaves were generally somewhat larger. The papillae on stems and ovaries were red in all plants as in *Franciscana*. The bud tips were all *Franciscana*-like and to a lesser degree the capsules. There was, therefore, in the culture no group of plants with wholly green stems and ovaries and no plants with foliage, bud tips and capsules *biennis*-like. There was, however, a wide range of variation in the flower size and in the position of the stigma whether high or low. My notes on the culture after the rosettes had become full sized and later when the plants reached maturity are presented in table 4 which may readily be compared with table 1.

Although I have grouped the plants in table 4 as *Franciscana*-like in most respects, this must not be understood to mean a perfect duplication of the parent in these characters. Leaves were generally larger than in *Franciscana* and capsules were frequently somewhat smaller, while bud tips varied in their length. Moreover, in flower size and in the position of the stigma the culture, *Franciscana*-like in other respects, presented many plants with the characters of *biennis*. There were, nevertheless, 6 plants in the culture essentially indistinguishable from *Franciscana* with respect to all of the characters discussed in this paper, the differences being small plus or minus expressions of the characters concerned.

The absence in this culture of a group of plants *biennis*-like in foliage, bud tips, and capsules was a surprise as was also the absence of green-stemmed plants representing a group of recessives from the dominant

TABLE 4

The chief segregates in culture 15,38, the F₂ of biennis × Franciscana E.

Mature rosettes	Papillae on stems and ovaries	Foliage at maturity	Bud tips	Capsules
Leaves narrower than <i>Franciscana</i> 17	Red 6	<i>Franciscana</i> -like in color and texture but narrower-leaved 6	<i>Franciscana</i> -like 4	<i>Franciscana</i> -like 4
<i>Franciscana</i> -like 186	Red 172	<i>Franciscana</i> -like 172	<i>Franciscana</i> -like 170	<i>Franciscana</i> -like 160
Intermediate between <i>biennis</i> and <i>Franciscana</i> 26	Red 24	<i>Franciscana</i> -like 24	<i>Franciscana</i> -like 20	<i>Franciscana</i> -like 17
Dwarfs, leaves sickle-shaped 15	Red 6	<i>Franciscana</i> -like 6	<i>Franciscana</i> -like 6	<i>Franciscana</i> -like 4
Other dwarfs which died as rosettes 3				
Totals 247	208	208	200	185

character of red papillae present in the F₁ generation. The only conspicuous suggestions in the culture of *biennis* characters were found in those rosettes intermediate in character between the parent types and in the flowers *biennis*-like as to the size of their petals and the position of their stigmas. I have no hypothesis to account for this one-sided expression of the culture so suggestive of *Franciscana*, but it should be emphasized that the high mortality in the culture, the large proportion of sterile seeds, and the partial sterility of the pollen indicate the probability of a very imperfect expression of the segregation which theoretically might be expected from the zygote of the F₁ parent plant. Until the significance of the gametic and zygotic sterility as well as the high mortality among the plants is understood it would be rash indeed to assume that segregation had not taken place during the development of the gametes at the end of the first hybrid generation. Therefore, that

which came to maturity in my cultures may have been from but a part of the range of zygotes formed and only a partial representation of the types theoretically possible if all of the gametes had been fertile among themselves.

TABLE 5

Position of stigma relative to the size of petals on plants which as rosettes were Franciscana-like; culture 15,38, the F_2 of biennis $\times$ Franciscana E.

Petals	Stigma high (Franciscana-like)	Stigma low (biennis-like)	Stigma intermediate	Totals
Large (Franciscana-like)....	6			6
Small (biennis-like).....	6	33	17	56
Intermediate	14	3	11	28
Smaller than biennis.....	3	1	2	6
Totals.....	29	37	30	96

The distribution of flower types on plants from rosettes which were *Franciscana*-like is shown in table 5. It will be seen that only 6 plants had large flowers and these also presented a high position of the stigma. Of 28 plants with petals intermediate in size between the parents only 3 had the stigma in a low position, and of the 56 plants with small flowers only 6 presented the stigma in the high position. The tendency, therefore, was for an association in the same flower of large petals with high stigmas and of small petals with low stigmas, but there were exceptions to this correlation.

TABLE 6

Position of stigma relative to the size of petals on plants which as rosettes were intermediate between biennis and Franciscana; culture 15,38, the F_2 of biennis $\times$ Franciscana E.

Petals	Stigma high (Franciscana-like)	Stigma low (biennis-like)	Stigma intermediate	Totals
Large (Franciscana-like)....	1		2	3
Small (biennis-like).....		10	1	11
Intermediate		4	1	5
Smaller than biennis.....		1		1
Totals.....	1	15	4	20

On 20 plants from rosettes intermediate between *biennis* and *Franciscana* (table 6), the flowers were mostly small or intermediate in size and the position of the stigma was generally low. Only 1 plant had large petals together with a high position of the stigma.

The group of dwarfs with sickle-shaped leaves was large and early recognized. They were similar to the dwarfs described in the previous culture and like them were vegetatively very weak. Only 6 out of the 44 produced shoots. I noticed in the development of the culture that many plants showed tendencies towards the production of sickle-shaped leaves, tendencies which were later outgrown, and this leads me to suggest that this dwarf form, as in the previous culture, may be the result of a diseased condition. It is a matter for further study.

HYBRIDS OF THE CROSS *FRANCISCANA* B $\times$ *BIENNIS* IN THE SECOND GENERATION,
FROM SEEDS GERMINATED IN THE EARTH

My observations on the F_2 hybrids of this cross, *Franciscana* B $\times$ *biennis*, were made in the summer of 1914, a year before the method of experimental seed-germination was developed. The cultures were from sowings in the earth, the seed-pans being kept for 8—11 weeks. There is, therefore, no assurance that all of the possibilities of the cultures were fully represented, since delayed germination undoubtedly held back a large part of the progeny. Consequently, the observations have little value as a genetical study of segregation and are only described because there were found in these cultures some interesting variants suggestive in certain respects of the so-called "mutants" from *Lamarckiana*. In this behavior of throwing occasionally exceptional forms these cultures presented a marked contrast to the F_2 from the cross *Franciscana* E $\times$ *biennis*, where, as we have seen, there was a clearly defined grouping of the types into a few large classes. From this difference in behavior when mated to the same line of *biennis* I am inclined to believe that the race *Franciscana* B is not so pure as the race *Franciscana* E. The cultures had their chief value as marking an important advance in the problem of synthesizing a *Lamarckiana*-like hybrid from a race of *Franciscana* pollinated by *biennis*, an account of which will be published at a later time.

The F_1 generation, 13.35, from which the cultures came, consisted of 165 plants and has already been described in detail (DAVIS 1914, pp. 188-192). It was more variable than that from the cross *Franciscana* E $\times$ *biennis* and five sister plants were selected for the parents of the five

selfed lines constituting the F_2 cultures, because of certain individual peculiarities as follows:

(1) Plant 13.35 *ac*, a type with smaller leaves, smaller flowers (petals 2 cm), and shorter stature than some plants of the culture. From this parent the F_2 culture 14.51 was grown, consisting of 386 plants.

(2) Plant 13.35 *b*, similar to plant 13.35 *ac* except for somewhat broader leaves and slightly larger flowers (petals 2.1 cm), the parent of the F_2 family 14.52 consisting of 255 plants.

(3) Plant 13.35 *c*, one of the largest-flowered types in the culture (petals 2.4 cm) and also large-leaved, the parent of an important F_2 culture 14.53 of 363 plants.

(4) Plant 13.35 *g*, similar to 13.35 *c* but with sepals at mid-summer entirely green, later taking on reddish streaks. From this parent came the F_2 culture 14.54 numbering 211 plants.

(5) Plant 13.35 *r* with sepals very red as in *rubrinervis*, and the mid-veins of the rosette leaves also a deeper red than in the mass of the culture, also with larger flowers (petals 2.4 cm) and larger leaves. This strongly colored type graded through intermediates to plants with the minimum of red as in 13.35 *g*. From it was grown the F_2 family 14.55 with 421 plants.

As was to be expected the families in the F_2 from the five sister plants listed above were distinguished in some important respects although having certain features in common. The points of agreement will be considered first.

In the five families constituting the total F_2 progeny 1636 plants sent up shoots upon which observations could be made respecting the color of the papillae over green portions of the stems. On all of these plants red papillae were found; none of them had the clear green stems of the *biennis* parent. Such results might lead one to suppose that there had been no segregation of this character (red of papillae) present in *Franciscana* and absent in *biennis*. Luckily there was left after the sowing of 1914 the contents of 8 capsules from the plant 13.35 *ac*. I was thus able in 1915 to obtain complete germinations in Petri dishes and thus to test the possibility that a class of recessives might be represented in seeds that required a longer time in the earth for their germination than the 8 to 11 weeks allowed in 1914.

The sowings of 1914 from plant 13.35 *ac* (culture 14.51) in earth produced 402 seedlings from 819 seed-like structures,—a germination of 49 percent. The sowings of 1915 of seeds from the same plant in a Petri dish (culture 15.51) gave 761 seedlings from 921 seed-like struc-

tures,—a germination of 82.6 percent. Thus the experimental method of seed-germination increased the product from the sowing 33.6 percent. The culture of 1914 from seeds sown in the earth produced from 402 seedlings 386 plants with shoots. Unfortunately the culture of 1915 from seeds germinated in Petri dishes was started so late in the hot-house that very many of the plants refused during the summer to pass from the rosette stage. Only 497 of the 762 seedlings produced plants

FIGURE 14.—Mature rosette of a plant, 14.51 *o*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana B* $\times$ *biennis*. Representative of a class distinguished by their rosettes of broad, obtuse-pointed leaves. Contrast with figure 16.

with shoots, but on 14 of these, a group of small-flowered types, I could find no red papillae. A group of green-stemmed plants thus appeared as in the case of the F_2 from the cross *Franciscana E* $\times$ *biennis* and again the experimental method of seed-germination justified itself as necessary for genetical research in the *oenotheras*.

The five families agreed closely as to the constitution of the mass of the cultures. There was present in each a well defined class distinguished as rosettes by their broad obtuse-pointed leaves (figure 14). Flowering

shoots from such rosettes presented a foliage of leaves and bracts also broad (figure 15) and small flowers of the *biennis* type with respect to the position of the stigma lobes below the level of the anther tips. The sepal tips were, however, thick and with a heavy pubescence like that of *Franciscana*. This class appeared in the five families in proportions of

FIGURE 15.—Inflorescence of a plant, 14.51 *o*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B $\times$ *biennis*. Representative of a class with a foliage of obtuse-pointed leaves, broad bracts, and small flowers of the *biennis* type but with thick sepal tips as in *Franciscana*. At the left is a leaf from near the base of the main stem. Contrast with figure 17.

41 percent (culture 14.51), 32 percent (14.52), 34 percent (14.53), 32 percent (14.54), and 39 percent (14.55), and these figures at least show that the class was numerically large. Curiously this class was not represented in the F_2 from *Franciscana* E $\times$ *biennis* (see table 1) which

shows that *Franciscana* B has a different genetical constitution from *Franciscana* E in spite of its taxonomic similarity.

The remainder of the plants in the five families had rosette leaves acute-pointed (figure 16) although of varying width and the leaves of the flowering shoots were narrow (figure 17) and likewise acute-pointed. This was a complex assemblage exhibiting a wide range in the form and size of the leaves. The flowers also ranged from those similar to *biennis*

FIGURE 16.—Mature rosette of a plant, 14.51 *p*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B $\times$ *biennis*. Representative of a group with rosette leaves acute-pointed. Contrast with figure 14.

to those of the *Franciscana* type and the sepal tips on some plants were thick like those of *Franciscana* and in others long and slender as in *biennis* and *Lamarckiana*. The assemblage undoubtedly contained several distinct classes distinguished by the characters of the bud tips and flowers, but data were not taken properly to differentiate them. I have records of 7 plants essentially similar to the *Franciscana* parent and there were undoubtedly more, but none of the *biennis* type were found since clear green stems together with typical *biennis* foliage were not repre-

sented in the cultures. It was in this assemblage that plants resembling *Lamarckiana* appeared.

Among the plants with acute-pointed leaves there appeared in all five families the same type of dwarf which has been described for the F_2 generations from the reciprocal crosses between *biennis* and *Franciscana* E. These dwarfs could be recognized as half-grown rosettes by their asymmetrical, sickle-shaped leaves (figure 18). Such rosettes always developed small plants which sent up irregularly disposed side branches generally not more than 2—4 dm long (figure 19). The leaves were broadly elliptical, the sepal tips *Franciscana*-like, and the flowers small (petals 1—1.7 cm). These dwarfs were not numerous in the cultures,

FIGURE 17.—Inflorescence of a plant, 14.51 *p*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B $\times$ *biennis*. Representative of a group with foliage of narrow, acute-pointed leaves and narrow bracts. At the right is a leaf from near the base of the main stem. Contrast with figure 15.

7 (14.51), 6 (14.52), 10 (14.53), 2 (14.54), and 3 (14.55), but it seems probable that a complete germination of the seeds would have given much larger proportions.

Some other types of dwarfs appeared but not uniformly in all of the five families. Culture 14.52 produced an *albida*-like plant and a very narrow-leaved dwarf both of which died early. In culture 14.53 were 3 small rosettes with leaves etiolated as in *albida* and a dwarf rosette with broader, obtuse-pointed leaves also etiolated; none of these lived

FIGURE 18.—Mature rosette of a dwarf with asymmetrical sickle-shaped leaves (culture 14.51). Representative of a group common in the F_2 generations from *Franciscana* $\times$ *biennis* and the reciprocal cross.

long. In culture 14.54 were 3 rosettes with leaves almost linear; they were fully green and lived through the summer but failed to produce shoots. In morphology these rosettes were very close to the figures and description of *Oenothera sublinearis* of DE VRIES, a "mutant" from *Lamarckiana* (Die Mutationstheorie, Vol. I, p. 285, 1901). A broad-leaved etiolated dwarf failed to develop beyond the rosette stage. Culture 14.55 had 9 etiolated dwarfs and a narrow-leaved plant all of which failed to survive the summer.

In the cultures was one plant, 14.51 *r*, worthy of special mention. Starting as a rosette with *Lamarckiana* characters it developed later a foliage of broad leaves (figure 20) but much shorter than in other broad-leaved forms in the cultures. The flowers were small (petals 1.1 cm) and in most cases the style and stigma withered to a brown filament rendering them infertile. This tendency towards the abortion of the style suggested the peculiarity of *brevistylis* although in other features

FIGURE 19.—Mature plant of a dwarf (culture 14.53) from a rosette with sickle-shaped leaves (figure 18). Representative of a group common in the F_2 generation from *Franciscana* $\times$ *biennis* and the reciprocal cross.

of its morphology there were no points of close resemblance to this plant. I was able to self a few flowers in which the style was not abortive and the progeny of this plant will be followed with interest.

One of the most interesting of the classes consisted of three plants

very conspicuous for their size and luxuriance. They are probably comparable to the triploid or *semi-gigas* forms from *Lamarckiana* (STOMPS 1912, LUTZ 1912), and *biennis* (STOMPS 1914, DE VRIES 1915 a), and also to the "hero" types from *Lamarckiana* or certain derivatives when pollinated by *leptomeris* (*biennis cruciata*), *muricata* or *Millersi* (DE VRIES 1913, p. 327). The plants of this giant or "hero" type appeared one in each of the cultures 14.52, 14.53, and 14.54. A description of the plant 14.52 g will serve for all. The extraordinarily large rosettes at maturity were 5.5 dm in diameter with leaves very broad and thick (figure 21). Side shoots from the rosettes (figure 22)

FIGURE 20.—Inflorescence of a plant, 14.51 r, in the F_2 from 13.35 ac (figure 9), *Franciscana* B $\times$ *biennis*. Peculiar for its foliage of short, broad leaves and for its small flowers in most cases with style and stigma withered to a brown filament rendering them infertile.

were unusually numerous (12 in 14.52 g, 11 in 14.54 g), widely spreading and very long (some 1—1.3 m). The stems were exceptionally strong, the leaves and bracts thick in texture. The buds (figure 23) were long (about 7.5 cm) and the cone stout although the flowers were not large (petals about 2.5 cm). The large stigma lobes were placed slightly below the level of the anther tips.

The evidence of gigantism exhibited by these plants suggested an increased number of chromosomes and a preliminary examination of mitotic figures in the developing ovules of plants 14.52 g and 14.54 g gave several counts certainly as high as 19 and 20 chromosomes so it seems probable that we have in these plants examples of triploidy (21 chromosomes). This view is further supported by the complete or al-

FIGURE 21.—Mature rosette of a plant, 14.52 g, in the F_2 from 13.35 b, *Franciscana* B $\times$ *biennis*. Remarkable for its very large, broad and thick leaves. Representative of three plants of a "hero" or *semi-gigas* type probably with the triploid number of chromosomes (21).

most complete self-sterility of the three plants, a condition characteristic of the triploid *semi-gigas* types that have been studied by other investigators. Attempts to self-fertilize numbered 21 for 14.52 g, 31 for 14.53 g, and 31 for 14.54 g. The yield was a few shriveled capsules containing some small structures of very doubtful viability which have not yet been sown. Attempts to pollinate the parent *biennis*, 28 from 14.52 g and 13 from 14.54 g, were all failures, and similar attempts to pollinate *Franciscana*, 11 from 14.52 g and 11 from 14.54 g, gave only a few

FIGURE 22.—Mature plant, 14.52 g, the *semi-gigas* type from the rosette shown in figure 21. Remarkable for its luxuriant growth as illustrated by the very long side shoots (some 1-1.3 m) from the rosette.

seeds(?) likely to prove sterile. In sharp contrast to the above experiments, flowers pollinated from the parents, *biennis* and *Franciscana*, developed capsules with a fair yield of apparently good seed, and open-pollinated flowers produced well filled capsules. Thus it appears clear that the self-sterility is not due to conditions within the ovules but must be concerned either with the development of pollen-grains, with a failure of pollen-tubes to reach the ovules, or to an inability of the gamete nuclei to fuse or to produce viable zygotes. A similar situation is pre-

sented in certain triploid hybrids of *gigas* with other *oenotheras* and offers an interesting cytological problem upon which I hope later to report.

There remain for consideration the plants which in certain respects resembled *Oenothera Lamarckiana*. They constituted a group selected at first because the mature rosettes (figures 24 and 25) closely matched those of *Lamarckiana*, and then observed throughout their development

FIGURE 23.—Inflorescence from the *semi-gigas* type, 14.52 g, (figures 21, 22), showing long buds with stout cones, but relatively small flowers (petals about 2.5 cm) with stigma lobes slightly below the level of the anther tips.

to establish whether or not as mature plants they continued to exhibit the peculiarities of this plant. Therefore with respect to the characters of the mature rosettes these plants constituted a group distinct from the obtuse-pointed, broad-leaved class (figure 14) and differing from other rosettes with acute-pointed leaves in having a leaf form and degree of

crinkling more close to that of *Lamarckiana*. The *Lamarckiana*-like rosettes were distributed among the five families as follows: 5 in 14.51, 1 in 14.52, 3 in 14.53, 2 in 14.54, and 13 in 14.55. These rosettes graded through intermediates into the large class with narrower-pointed leaves, illustrated by figure 16, which constituted more than one-half of the total number of plants in the cultures.

In culture 14.51, as stated above, there were 5 rosettes *Lamarckiana*-like in the form, size, and crinkled surface of their leaves. Of these plants 4 sent up shoots with a foliage also of the *Lamarckiana* type, but the flowers were all small (petals about 2 cm long) and the stigma lobes were close to the level of the anther tips, in some plants slightly above and in some slightly below. The 4 plants were of a type very similar to some of the small-flowered forms of *Lamarckiana* which I have obtained from seed of DE VRIES'S (DAVIS 1912, p. 384) and from seed of English origin (DAVIS 1913 b, p. 237).

The single plant of culture 14.52, selected as being conspicuously *Lamarckiana*-like as a rosette, also proved at maturity to be a small-flowered type.

FIGURE 24.—A *Lamarckiana*-like rosette, plant 14.55 ac, in the F_2 from 13.35 r, *Franciscana* B $\times$ *biennis*.

Culture 14.53 gave what is likely to prove the most valuable of the *Lamarckiana*-like forms of the cross. A plant, 14.53 c, presented fairly large flowers (petals 3.3 cm) with stigma lobes 2—3 mm above the tips of the anthers and, what is very important for the synthesis of a *Lamarckiana*-like hybrid, short capsules (1.8—2 cm long), in form, size, and pubescence quite indistinguishable from those of *Lamarckiana*. As the plant stood in this F_2 generation it seemed to me to fall within the limits of DE VRIES's account of the variation present in *Lamarckiana*,

FIGURE 23.—A *Lamarckiana*-like rosette, plant 14.55 c, in the F_2 from 13.35 r, *Franciscana* B $\times$ *biennis*. Failing to send up the central shoot this rosette became unusually large.

although the plant did not quite match the largest-flowered races that he has distributed (petals 4—4.5 cm), races which appear to be the élite among the *Lamarckianas*. Among the progeny of this selfed plant grown last summer (1915) in the F_3 I have had types with larger flowers (petals 3.7 cm) and in all other important respects essentially similar to *Lamarckiana*. These will be described in detail probably after

the F_4 has been grown. The two other plants of culture 14.53, *Lamarckiana*-like in foliage, proved to be small-flowered.

The two *Lamarckiana*-like rosettes in culture 14.54 failed to send up shoots and did not survive the winter.

The 13 *Lamarckiana*-like rosettes of culture 14.55 fell into two groups. In 6 of these rosettes the mid-veins were white as is true of the races of *Lamarckiana* familiar to me; in 7 of the rosettes the mid-veins were reddish as described by HERIBERT-NILSSON (1912, 1915) for certain material that he has handled. The presence in the culture of white-veined rosettes is of interest for the reason that the rosette leaves of both *biennis* and *Franciscana*, as far as I have observed, always show more or less red pigmentation in their mid-veins, as was also true of their F_1 hybrids. The amount of red pigmentation varied greatly among the plants of the F_2 generations and I very much doubt whether the character could be followed with precision in the hybrids of my cultures.

Of the 6 *Lamarckiana*-like rosettes with white mid-veins, 4 sent up side branches that flowered and the foliage and bracts of these shoots (figure 26) presented the characters of *Lamarckiana*. Since no central shoots developed, the rosettes grew to be very large (figure 25) evidently adopting a biennial habit, as *Lamarckiana* frequently does under certain conditions. When the plants came to bloom, 3 bore large flowers (petals 3.5 cm) with the stigma lobes 3 mm or more above the tips of the anthers; the other plant was small-flowered (petals 1.5 cm) and the stigma lobes were 1—2 mm below the anther tips. The larger-flowered plants (figure 26) were among the most beautiful in the cultures. Their foliage was very close to that of *Lamarckiana* and the pubescence was similar, but the buds proved to be more slender, the inflorescence less compact, and the capsules (2.5 cm) too long and attenuate to match the type of *Lamarckiana* known to us from seeds of DE VRIES, although the capsules fell both as to form and size within the range of variation given by him ("Die Mutationstheorie", Vol. I, pp. 337—339, figure 114, 1901). I have grown an F_3 generation from one of these plants and while I obtained larger flowers (petals 4 cm) and a type of inflorescence more like that of *Lamarckiana* there were no plants with short capsules.

Of the 7 *Lamarckiana*-like rosettes with red mid-veins, 5 sent up flowering side branches with foliage and bracts similar to *Lamarckiana*, but all of the plants were small-flowered (petals about 2 cm long), as in culture 14.51.

HYBRIDS OF THE CROSS *BIENNIS* × *FRANCISCANA* B IN THE SECOND GENERATION, FROM SEEDS GERMINATED IN THE EARTH

A culture (14.56) was grown from seeds of the F_1 hybrid plant 13.36 *a*, *biennis* × *Franciscana* B (figure 10), described in my earlier paper (DAVIS 1914, pp. 190, 191). The sowing was made in earth, seed-pans being kept for 8—10 weeks, and the germination was very poor since from 637 seed-like structures (contents of 4 capsules) only 54 seedlings appeared, a germination of 8 percent. That this unsatisfactory result was due to delayed germination has since been established by experimental germination of more seeds from the same parent plant, 13.36 *a*, when 438 seedlings (culture 15.52) resulted from 623 seed-like structures, a germination of 70 percent.

Of the 54 seedlings, 51 developed rosettes which sent up flowering shoots during the summer. Since this culture was but a small fraction of the possibilities in the seeds sown, it obviously has no genetical value, and I shall only record its general character and the presence of one remarkable variant.

The culture was very uniform in the habit and foliage of the plants which resembled closely the parent F_1 hybrid 13.36 *a* (figure 10). They were large plants with narrow leaves of the *Franciscana* form and texture but larger. The pubescence was also like that of *Franciscana*, all plants having red papillae on the stems and ovaries. The buds had the thick sepal tips and pubescence of *Franciscana*, but the flower size varied from petals 1.1 to 2.8 cm in length. Most of the flowers were very close to the form and size presented in the F_1 parent hybrid.

A remarkable variant, 14.56 *w*, appeared with all flowers having cream-white petals (2.8 cm long); in other respects it resembled the other plants of the culture. This striking modification of color seems to parallel the change which takes place when the color variety *sulfurea* is thrown off from the Dutch *Oenothera biennis* as described by STOMPS (1914) and DE VRIES (1915 *a*). That the *biennis* parent of my cross should also have come from Dutch lines adds to the interest in this remarkable plant. The plant when selfed set an abundance of seed and should prove an important form for future study.

DISCUSSION AND SUMMARY

The chief interest in the results here presented lies in the clear evidence that an F_2 generation of a species cross of *Oenothera* may produce several classes sharply distinguished from one another and without inter-

grading types. The studies have an especial value since the germination of seeds in some of the cultures was experimentally determined to be complete. They are the first cultures of this character to be described and although the mortality among the seedlings was great it seems probable that the cultures were much more largely representative of their genetical possibilities than when in past years seeds have been sown in the earth.

This behavior, i.e., the production of classes in the progeny of hybrid *oenotheras*, has been described in the writings of several students of *Oenothera* genetics. Thus the twin and triple hybrids discovered by

FIGURE 26.—Inflorescence from the plant 14.55 *ac*, one of the most beautiful of the F_2 hybrids from *Franciscana B* $\times$ *biennis*. *Lamarckiana*-like as to flowers and foliage and as a rosette (figure 24), but with inflorescence less compact and buds more slender than in *Lamarckiana*. At the right is a leaf from the large rosette.

DE VRIES 1907, 1909), although appearing as the products of direct crosses, may in reality be of generations later than an F_1 if either or both of the parents were hybrid in constitution. The studies of the author on hybrids between *grandiflora* and a form of "American *biennis*" (DAVIS 1912, 1913) clearly indicated the differentiation of classes in the F_2 as well as phenomena apparently parallel to the production of twin hybrids in certain F_1 generations, the latter situation indicating that the "*biennis*" parent type was impure. The observations of ATKINSON (1914) on twin and triple hybrids of *Oenothera nutans* and *Oe. pycnocarpa* seem to furnish another illustration of segregation following a direct cross between parents of doubtful genetic purity. The interesting correlations of pigment distribution reported by SHULL (1914) in crosses of *rubricalyx* with *Lamarckiana* and *rubrinervis* likewise suggest segregation of characters from complex material. And the studies of GATES (1914) on hybrids between *grandiflora* and *rubricalyx* seem to the writer inconclusive for his contention that the behavior is non-Mendelian, when it is recognized how incomplete may have been the representation of the genetical possibilities in his frequently small and earth-sown cultures. The uncertainty of complete representations or of correct ratios which attaches to all of this earlier work, because of the failure to allow for the probabilities of incomplete germination and for the factors of gametic and zygotic sterility, have been sufficiently emphasized in the introduction.

The most striking of my results were exhibited by the F_2 of the cross *Franciscana* E $\times$ *biennis* in cultures where seed-germination was complete. If table I be examined with reference to a number of characters several interesting correlations which have the appearance of linkage, will become evident. (1) There was a large group of plants (182) *Franciscana*-like as to rosettes, stem coloration, foliage, bud tips and capsules, and a smaller group (16) similar to this but with narrower leaves. (2) A large group (67) *biennis*-like in rosettes, foliage, bud tips and capsules had, however, the stem coloration of *Franciscana*. (3) Green stems as in *biennis* were found only in a group (24) with rosette leaves obtuse-pointed and with foliage *Franciscana*-like but broader-leaved, and with buds and capsules also *Franciscana*-like. (4) A group (35) with evanescent rosettes contained a large number of plants (29) *Franciscana*-like and a small number (4) *biennis*-like in foliage, bud tips and capsules, while all of the plants had the stem coloration of *Franciscana*. (5) The dwarfs (59) with rosette leaves sickle-shaped were likewise mostly *Franciscana*-like (36), but some resembled *biennis* (3) in

foliage, bud tips and capsules although all agreed in having the stem coloration of *Franciscana*. This seems to me evidence clear-cut for the presence of distinct classes in the culture and I can see no reasonable interpretation except on the basis of a process of segregation determining different types of gametes at the end of the first hybrid generation, a behavior Mendelian in its fundamental nature.

The distribution of floral characters with reference to the classes which as to foliage were either *Franciscana*-like or *biennis*-like is given in tables 2 and 3. There is clearly a tendency to link within the same flower large petals or petals intermediate in size with a high position of the stigma, and conversely small petals were generally associated with a low or intermediate position of the stigma. However, while most of the *Franciscana*-like plants had large flowers and most of the *biennis*-like plants small flowers, there were numerous exceptions to the rule and no fixed association was discovered. It will be remembered that DE VRIES (1911, 1913) reported similar ranges of variation in flower size in hybrids patroclinous or matroclinous with respect to vegetative characters.

A remarkable feature of this cross (*Franciscana* E $\times$ *biennis*) in the F_2 was its clear segregation with respect to foliage into two groups, one resembling the *Franciscana* parent (274 plants) and the other the *biennis* type (70 plants). All of the data obtained on bud tips and capsules (see table 1) indicate that these characters of the parents also followed in their segregation the behavior of the foliage. The situation thus indicates that a large group of characters are closely correlated and their factors in inheritance are apparently closely linked. It is interesting to note that the differences in foliage characters bear a marked resemblance to the peculiarities that separate the twin hybrids, *laeta* and *velutina*, discovered by DE VRIES (1907) when *Lamarckiana* and certain of its "mutants" were crossed with *biennis* and some other forms. The hypothesis of RENNER (1914) that *Lamarckiana* is a heterozygote of a cross involving *laeta* and *velutina* forms takes on added interest from the behavior of the F_1 hybrids of *Franciscana* $\times$ *biennis*, for these species with respect to foliage characters have respectively the *laeta* and *velutina* characteristics. I shall cross certain *Lamarckiana*-like hybrids of *Franciscana* B $\times$ *biennis* with the Dutch *biennis* and with DE VRIES's "*biennis* Chicago" to determine whether they will give a twin progeny.

Among the *Franciscana*-like plants were a large number (about 52), which in all of their characters so closely resembled the *Franciscana* parent as to be virtually indistinguishable by taxonomic tests. Among

the plants *biennis*-like in foliage were 33 individuals with rosettes, bud tips, flowers, and capsules also as in the *biennis* parent, but none of these had green stems and ovaries (without red papillae) and consequently there were no segregates that completely matched the *biennis* type. Apparently factors for green stems either fail to link during segregation with other *biennis* factors, or if sometimes they do so segregate, the zygotes are not fertile. At least in a fairly large culture all of the 23 green-stemmed plants had foliage characters of *Franciscana*. There is indicated by these facts a high degree of correlation among the characters and it seems probable that much of the inheritance in *oenotheras* will exhibit extensive linkage of factors such as is known in other groups.

The F_2 from the reciprocal cross, *biennis* $\times$ *Franciscana* E, also from seeds completely germinated, gave a smaller number of classes than in the F_2 from the cross *Franciscana* E $\times$ *biennis*, but there was present a similar correlation of characters indicating extensive linkage of factors. As shown in table 4 the culture consisted of (1) a large group (186) *Franciscana*-like in rosettes, stem coloration, foliage, bud tips and capsules together with a small group (17) similar except for narrower leaves; (2) a group intermediate between the parents as to rosette characters but *Franciscana*-like in other respects; and (3) a group of dwarfs with sickle-shaped rosette leaves but also *Franciscana*-like in other characters. There were thus in the culture no classes *biennis*-like in rosettes, stem coloration, foliage, bud tips or capsules, and the only conspicuous suggestions of this parent appeared in rosettes intermediate in character and in the flowers more frequently small and with a low position of the stigma. The culture was therefore distinctly one-sided and *Franciscana*-like in the expression of its vegetative characters but the high degree of sterility, gametic and zygotic, may account for this fact through the elimination of types which theoretically would be expected as the result of a normal segregation from such a cross. On the assumption that forms *biennis*-like in various respects were thus eliminated the surviving groups may be held to represent one side (*velutina*) of a segregation theoretically probable. The seed-fertility of only 57 percent, the extensive pollen-sterility, and the high mortality in the culture are ample to allow of such a possibility.

It is curious that this culture, generally *Franciscana*-like in vegetative expression, should for the most part have had small flowers or flowers in size intermediate between the parents, yet an examination of tables 5 and 6 will show this to have been the case. With the small flowers was generally associated a low position of the stigma. However, 6

plants, *Franciscana*-like in vegetative characters, also had large flowers with a high position of the stigma and these plants were essentially indistinguishable from the *Franciscana* parent.

Neither of the two cultures presented flowers appreciably larger than those of the *Franciscana* parent, but there were 15 plants in the F_2 from *Franciscana* E $\times$ *biennis* and 7 in the F_2 from the reciprocal the flowers of which were smaller than the *biennis* type. The petals of these plants were from 9—15 mm long and generally crumpled; the plants in other respects seemed normal and set seed abundantly. Future cultures will determine whether or not this character is germinal.

There are also described in this paper hybrids in the F_2 from a cross between another line of *Franciscana*, designated as *Franciscana* B, and the same race of *biennis*. The cultures, grown in 1914, were from seed germinated in the earth and undoubtedly only imperfectly indicated the genetical possibilities of the cross, but they, nevertheless, presented a greater variety of types in the F_2 than were found in the crosses between *Franciscana* E and *biennis*. The indications are clear that *Franciscana* B in spite of its taxonomic resemblance to *Franciscana* E has not in reality quite the same germinal constitution.

Cultures in the F_2 of *Franciscana* B $\times$ *biennis* were grown from seeds of 5 sister F_1 plants which differed in relatively small particulars and each of the 5 families from these F_1 parent plants exhibited its own characteristics. Consequently the F_1 generation was clearly made up of somewhat diverse biotypes, a condition not uncommon in species crosses and which I also found in my studies on hybrids between *grandiflora* and an "American *biennis*" (DAVIS 1912, 1913).

The most interesting forms in the F_2 from the cross *Franciscana* B $\times$ *biennis* were the following: (1) A number of plants appeared very similar to *Lamarckiana* in vegetative characters and some of these bore large flowers. One individual, 14.53 c, with large flowers and short capsules has been selected as the starting-point of a line already taken through the F_3 in the expectation that a *Lamarckiana*-like hybrid may be synthesized. (2) Probable triploid types (chromosomes counted up to 19 and 20), comparable in their size and self-sterility to the *semi-gigas* derived from *Lamarckiana* and *biennis*, were represented by 3 plants which appeared each in a different family. (3) A single plant, 14.51 r, with a foliage of broad leaves bore flowers in most cases with style and stigma withered to a brown filament rendering them infertile, a condition suggestive of the chief peculiarity of *brevistylis*. (4) A large number of dwarfs with acute-pointed, sickle-shaped rosette leaves

corresponded to a similar group in the F_2 from the cross *Franciscana* E $\times$ *biennis*. (5) Etiolated dwarfs similar to DE VRIES'S *albida* were represented by 4 rosettes which died early, and there were also 3 rosettes with green leaves almost linear, which resembled *sublinearis* DE VRIES.

The mass of the F_2 cultures consisted of two groups distinguished as rosettes. The first of these had rosette leaves broad and obtuse-pointed, a foliage of broad leaves, and small flowers of the *biennis* type except that the bud tips were *Franciscana*-like; these plants constituted from 32—41 percent of the cultures. The second group was less uniform but had rosette and foliage leaves narrow and acute-pointed, flowers that in form and size ranged between those of the parents, and sepal tips in some plants thick as in *Franciscana* and in others long and slender as in *biennis* and *Lamarckiana*. There was probably material here for the differentiation of a number of classes but my data is too imperfect to discuss this point.

A number of plants were noted taxonomically indistinguishable from the *Franciscana* parent, but none that had all of the characters of *biennis*. Segregates of the *biennis* type were absent by virtue of the fact that all of the plants in the culture had the stem coloration (red papillae) of the *Franciscana* parent. That a class of green-stemmed plants was represented in the zygotes seems to be established by another culture grown a year later (1915) from sister seeds germinated experimentally. In this culture a group of 14 green-stemmed, small-flowered plants appeared, but this culture, started late in the season, failed to send up many shoots and the data from it has little genetical value.

The dwarfs, distinguished as rosettes by their sickle-shaped leaves, which appeared in these F_2 cultures as well as those from the crosses between *Franciscana* E and *biennis* deserve brief discussion. There was much variation in the extent of the asymmetrical development shown by the leaves, a character which has been reported in other cultures of *Oenothera* (see GATES 1913, pl. I, fig. 12, *Oe. Lamarckiana* var. *cruciata*), and is not infrequently shown to a greater or less degree in the rosettes of *Oenothera nanella* DE VRIES. I have noted that occasionally from such rosettes full-sized normal plants may develop instead of the stunted irregular growths generally present. This opens the question whether or not the sickle-leaved dwarfs really constitute a class distinguished by a particular genotype or whether their peculiarities may not be due to a diseased condition. Further genetical studies will be necessary to determine this point.

The F_2 generation from the reciprocal cross *biennis* $\times$ *Franciscana* E

was represented by a very small culture due to delayed germination. It was chiefly interesting for the appearance of a plant with cream-colored petals, a color variety which apparently parallels the form *sulfurca* thrown by *biennis*.

In conclusion, the results of these studies on hybrids between *Oenothera biennis* and *Oe. Franciscana* appear to the writer to give positive evidence of a segregation of factors in the F_2 generation of a character to be expected in Mendelian inheritance. The differentiation of the classes in the F_2 was unmistakable and a remarkable feature of these classes was the firm correlation of many characters indicating a remarkable linkage of certain factors. That the situation, if Mendelian, is very complex becomes apparent from the extent of the linkage. Although certain of the cultures, because grown from seed forced to a complete germination, are probably fairly representative of the genetical possibilities of the F_1 hybrid parents it must be remembered that very large numbers of plants either died or could not be brought to maturity. Furthermore, there was a large amount of sterility in the pollen (about 50 percent) and to almost as great a degree in the seeds produced by the F_1 generation. For these reasons the author has refrained from speculation on the possible ratios that might be suggested for the appearance of classes or of characters on Mendelian hypotheses. The high degrees of sterility both gametic and zygotic present problems of irregularities so great as to render speculation at present most unsafe. Also, the parent species have not themselves passed the tests of a pure species (DAVIS 1915 b), and there are suggestions in the facts of seed-sterility in *Franciscana* and of pollen-sterility in *biennis* that these species are not strictly homozygous. Mendelian studies on the *oenotheras* seem to the writer at present to be quite hopeless except as they are concerned with the simplest conditions that can be experimentally devised and deal with parent material exhaustively tested for its genetic purity or of which the genotypes are most thoroughly known.

University of Pennsylvania
December 1915

LITERATURE CITED

- ATKINSON, G. F., 1914 Segregation of unit characters in the zygote of *Oenothera* with twin and triplet hybrids in the first generation. *Science* **39**: 834-835.
BARTLETT, H. H., 1913 The delimitation of *Oenothera biennis* L. *Rhodora* **15**: 48-53.
1914 *Oe. Franciscana* and *Oe. venusta* spp. novo, *Rhodora* **16**: 33-37.
1915 a Additional evidence of mutation in *Oenothera*. *Bot. Gaz.* **59**: 81-123.
1915 b The mutations of *Oenothera stenomeris*. *Amer. Jour. Bot.* **2**: 100-109.

- 1915 c Mutations *en masse*. Amer. Nat. **49**: 129-139.
- 1915 d Mass mutation in *Oenothera pratincola*. Bot. Gaz. **60**: 425-456.
- DAVIS, B. M., 1912 Further hybrids of *Oenothera biennis* and *Oe. grandiflora* that resemble *Oe. Lamarckiana*. Amer. Nat. **46**: 377-427.
- 1913 a The behavior of hybrids between *Oenothera biennis* and *Oe. grandiflora* in the second and third generations. Amer. Nat. **47**: 449-476, 547-571.
- 1913 b The problem of the origin of *Oenothera Lamarckiana*. New Phytol. **12**: 233-241.
- 1914 Some reciprocal crosses of *Oenothera*. Ztschr. f. ind. Abst. u. Vererb. **12**: 169-205.
- 1915 a A method of obtaining complete germination of seeds in *Oenothera* and of recording the residue of sterile seed-like structures. Proc. Nat. Acad. Sci. **1**: 360-363.
- 1915 b The test of a pure species of *Oenothera*. Proc. Amer. Phil. Soc. **54**: 226-245.
- 1915 c Additional evidence of mutation in *Oenothera*? Amer. Nat. **49**: 702-706.
- DE VRIES, H., 1907 On twin hybrids. Bot. Gaz. **44**: 401-407.
- 1909 On triple hybrids. Bot. Gaz. **47**: 1-8.
- 1911 Ueber doppeltreziproke Bastarde von *Oenothera biennis* L. und *Oenothera muricata* L. Biol. Centralbl. **31**: 97-104.
- 1913 Gruppenweise Artbildung. pp. viii + 365. Berlin: Gebrüder Borntraeger.
- 1915 a The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. **59**: 169-196.
- 1915 b Ueber künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**: 161-176.
- 1915 c *Oenothera gigas nanella*, a Mendelian mutant. Bot. Gaz. **60**: 337-345.
- EAST, E. M., 1912 The Mendelian notation as a description of physiological facts. Amer. Nat. **46**: 633-655.
- GATES, R. R., 1913 A contribution to a knowledge of the mutating *oenotheras*. Trans. Linn. Soc. Botany **8**: 1-67.
- 1914 Breeding experiments which show that hybridization and mutation are independent phenomena. Ztschr. ind. Abst. u. Vererb. **11**: 209-279.
- 1915 a The mutation factor in evolution with particular reference to *Oenothera*. pp. xiv + 353. London: Macmillan and Co.
- 1915 b On successive duplicate mutations. Biol. Bull. **29**: 204-220.
- HERIBERT-NILSSON, N., 1912 Die Variabilität der *Oenothera Lamarckiana* und das Problem der Mutation. Ztschr. ind. Abst. u. Vererb. **8**: 89-232.
- 1915 Die Spaltungserscheinungen der *Oenothera Lamarckiana*. Lunds Universitets Arsskrift N. F. **12**: 1-132.
- LUTZ, ANNE M., 1912 Triploid mutants in *Oenothera*. Biol. Centralbl. **32**: 385-435.
- RENNER, O., 1914 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora **107**: 115-150.
- SHULL, G. H., 1914 A peculiar negative correlation in *Oenothera* hybrids. Jour. Gen. **4**: 83-102.
- STOMPS, T. J., 1912 Die Entstehung von *Oenothera gigas* de Vries. Ber. deutsch. bot. Gesell. **80**: 406-416.
- 1914 Parallele Mutationen bei *Oenothera biennis* L. Ber. deutsch. bot. Gesell. **32**: 179-188.